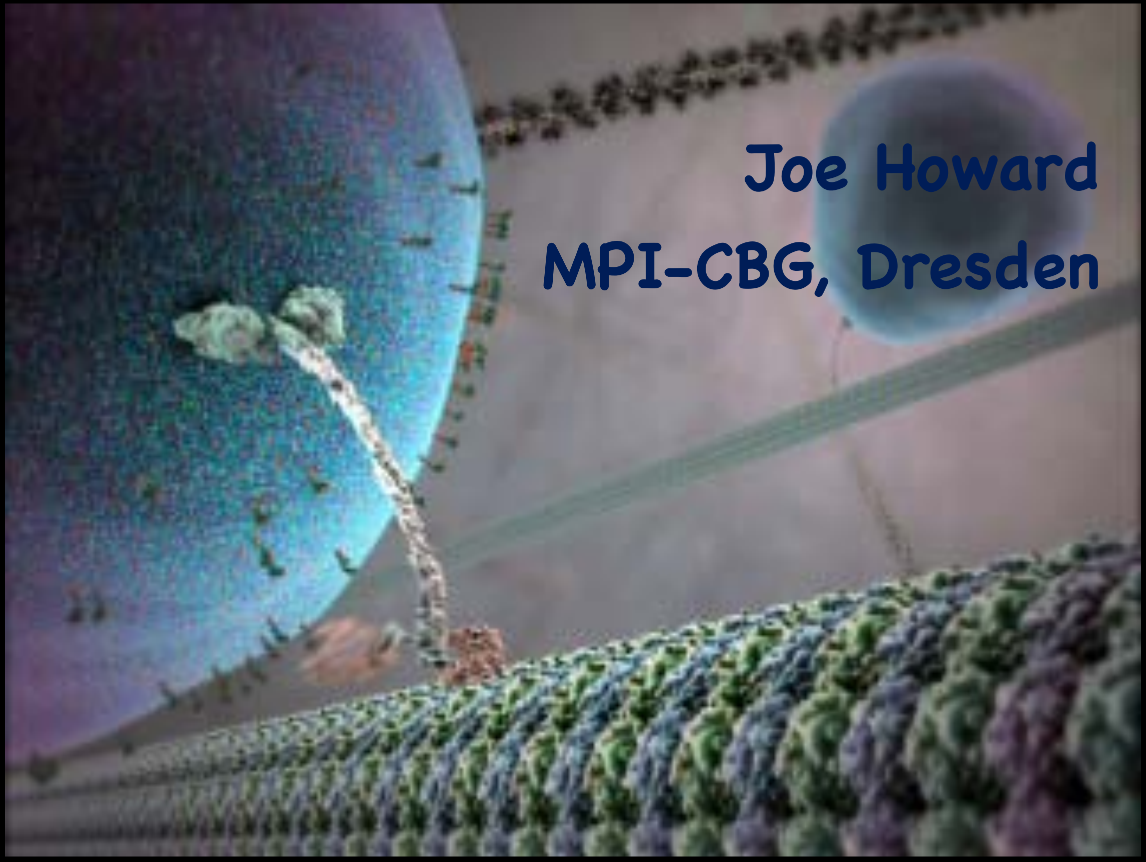
Max Planck Institute of Molecular Cell Biology and Genetics

ICTS-NESP
Kanpur
2 February 2010

Motor Proteins as Nanomachines: Force, Friction and Fluctuations



Joe Howard
MPI-CBG, Dresden

The inner life of the cell (Harvard Univ.)

MAX-PLANCK-GESELLSCHAFT

Outline

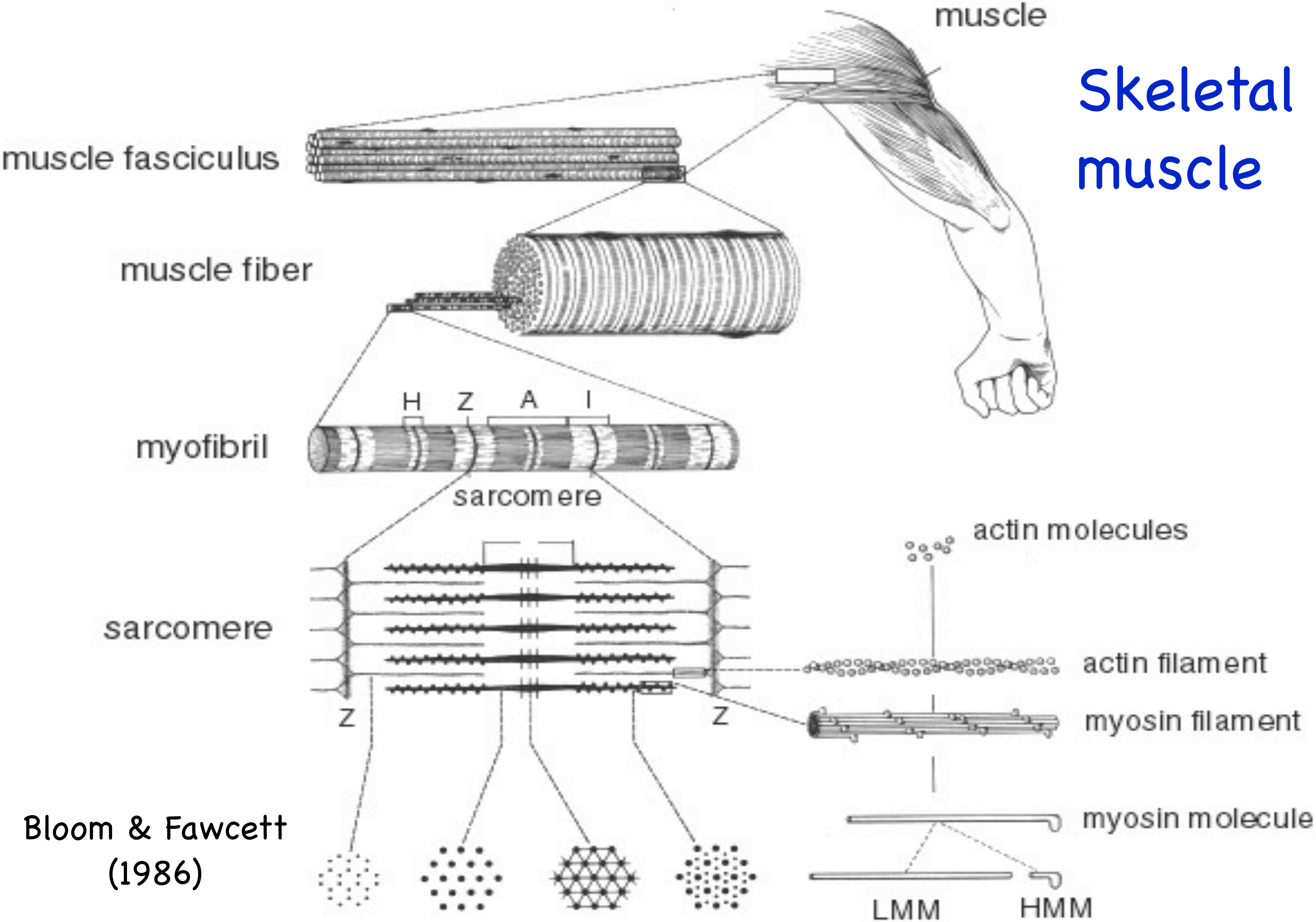
1. **Single-molecule techniques** can be used to study movement of purified motor proteins

2. Role of **fluctuations** in the motor reaction

3. **Protein friction** limits motor speed and efficiency

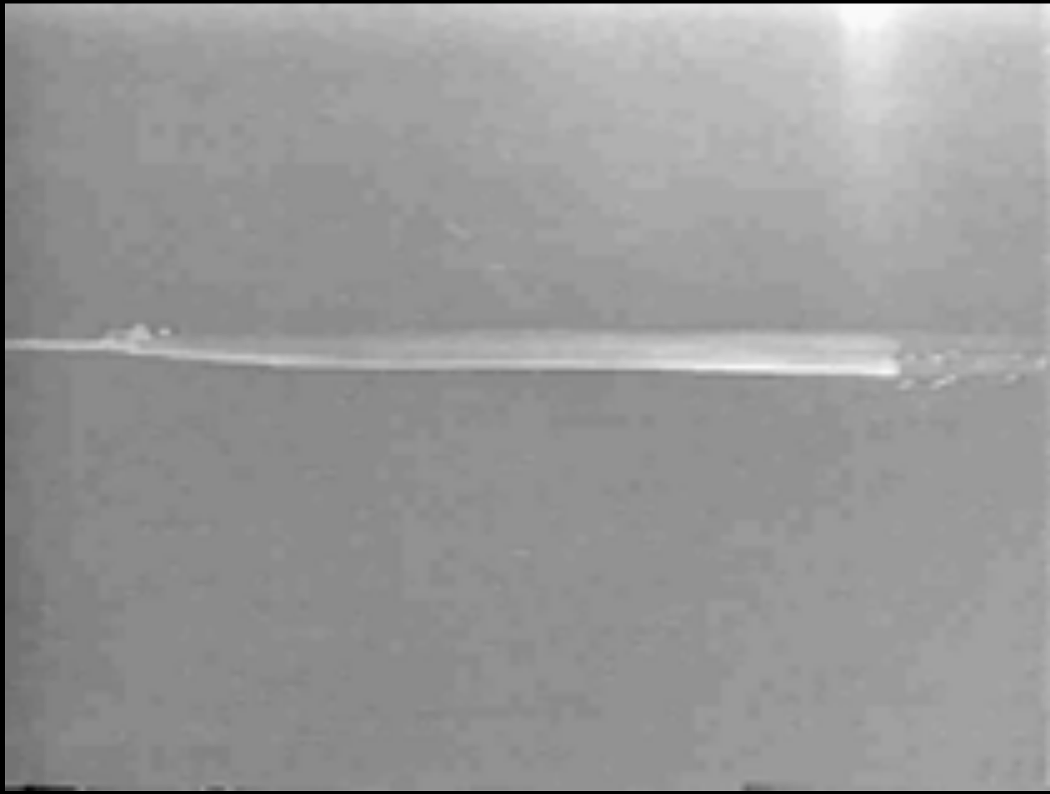
4. **Force gating of motor proteins:** active mechanical circuits underlying cell motility

Skeletal muscle

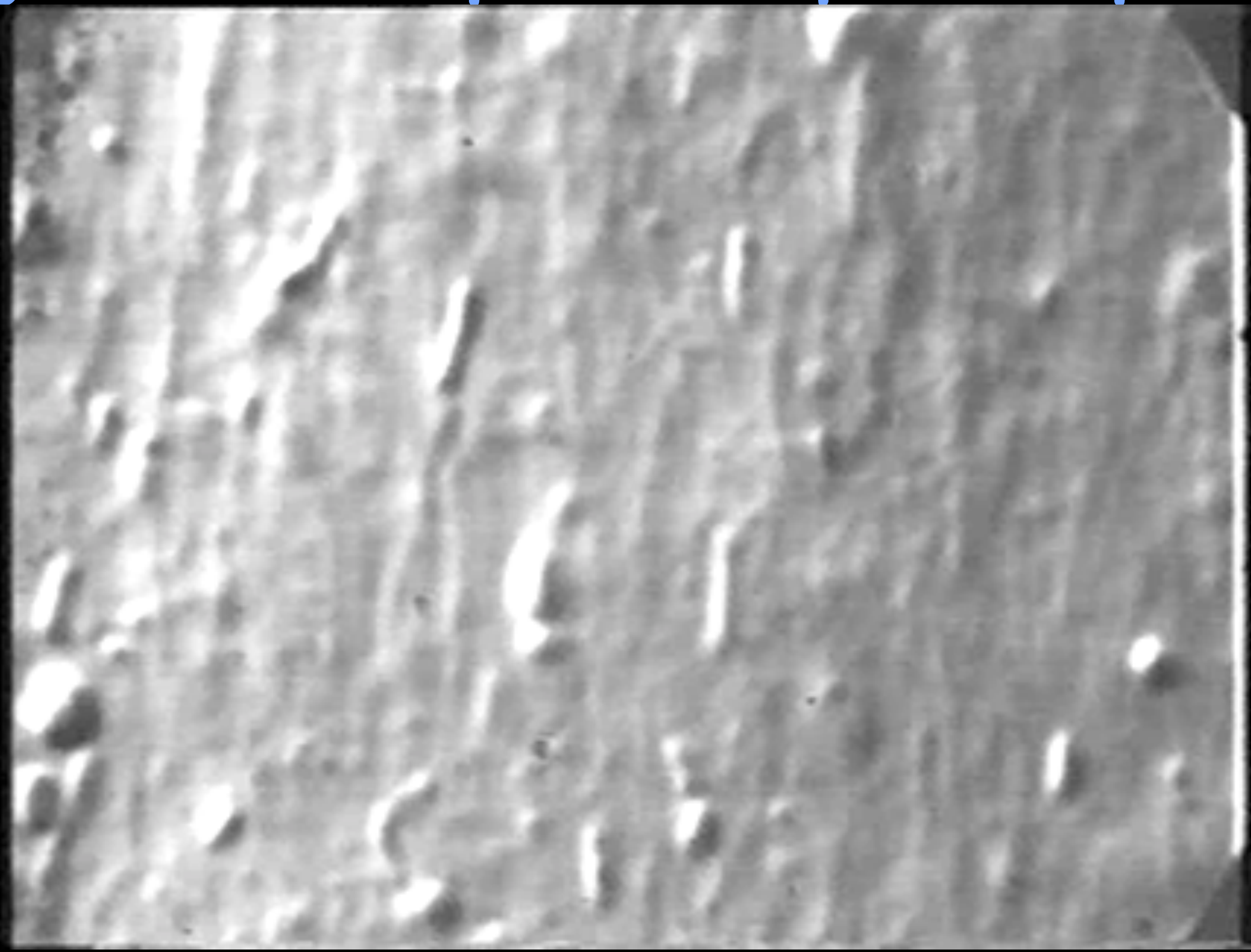


Bloom & Fawcett
(1986)

Squid giant axon



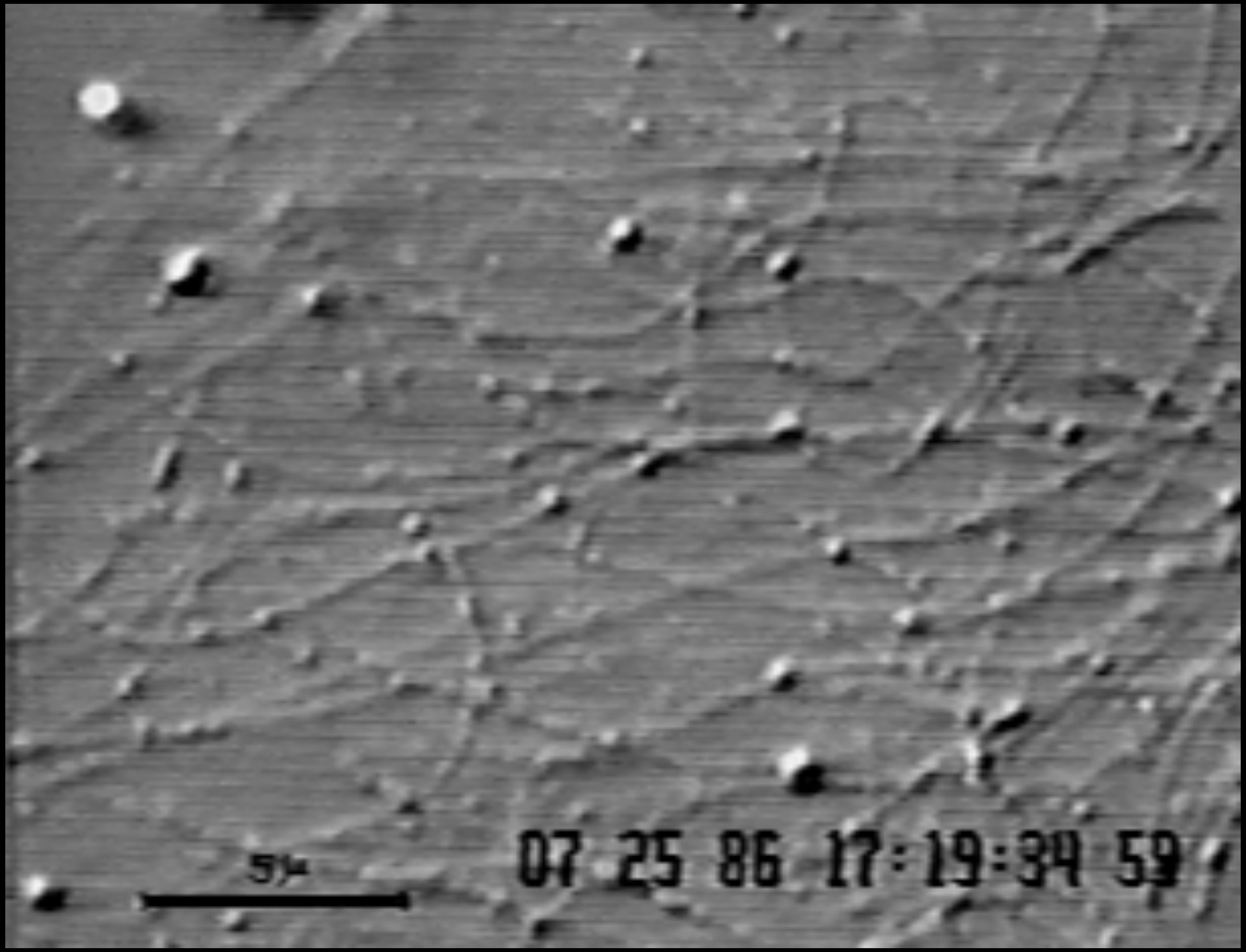
Organelle transport in squid axoplasm



10 µm

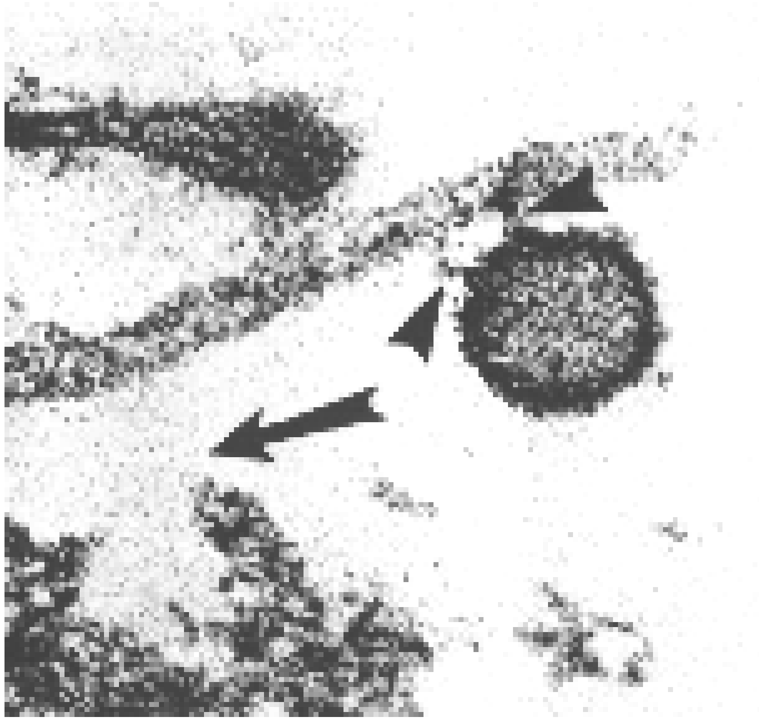
Robert Allen, Woods Hole

Organelle transport in squid axoplasm

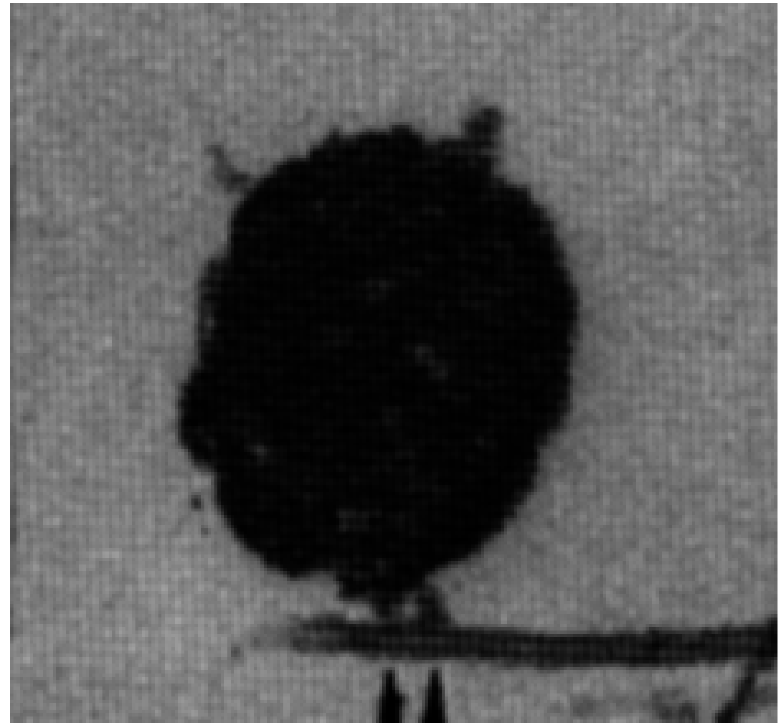


Dieter Weiss, Rostock

Crossbridges between microtubules and organelles



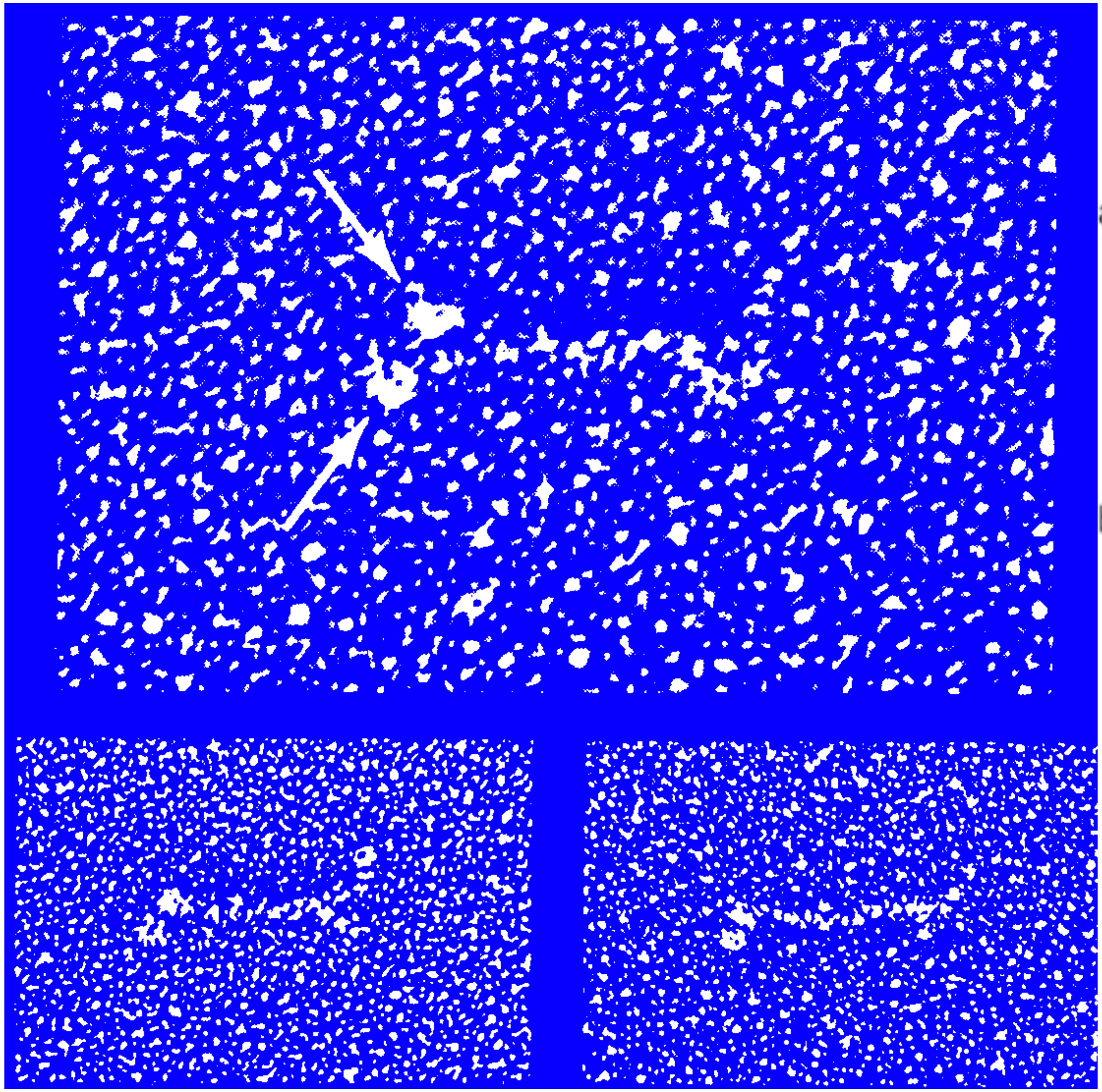
100 nm

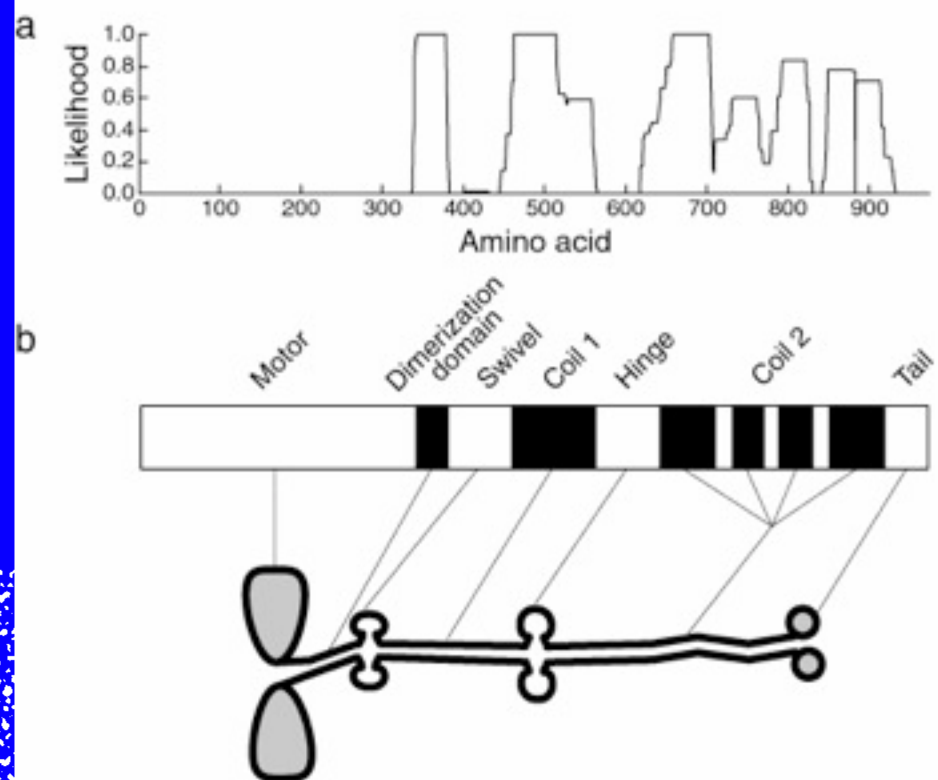


Miller & Lasek, J. Cell Biol. 101: 2181 (1985)

Ashkin et al., Nature 348: 346 (1990)

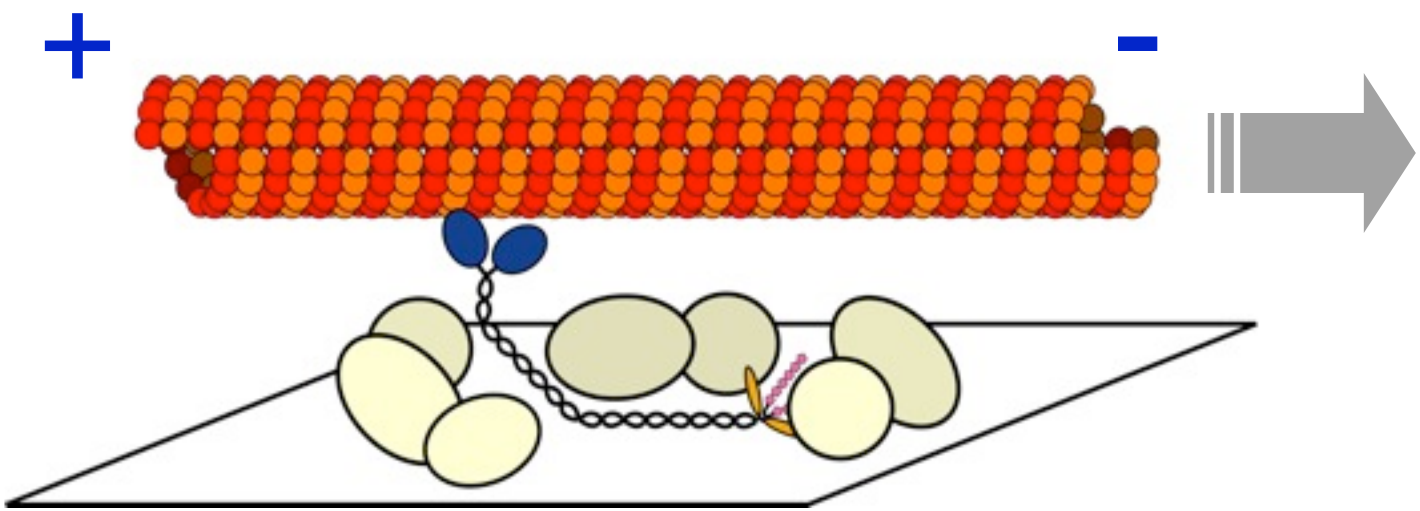
Kinesin



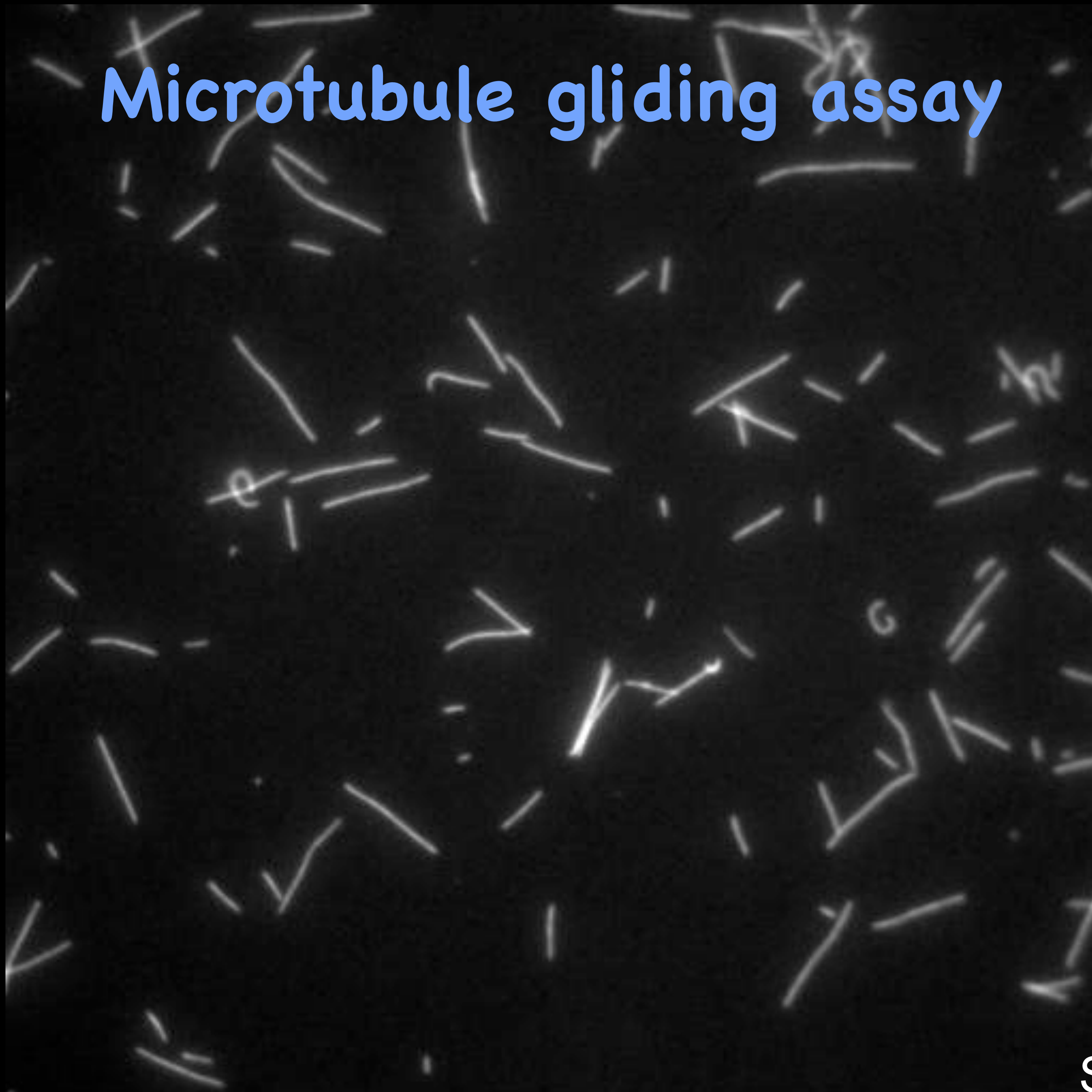


Hirokawa et al., Nature 348: 346 (1990)

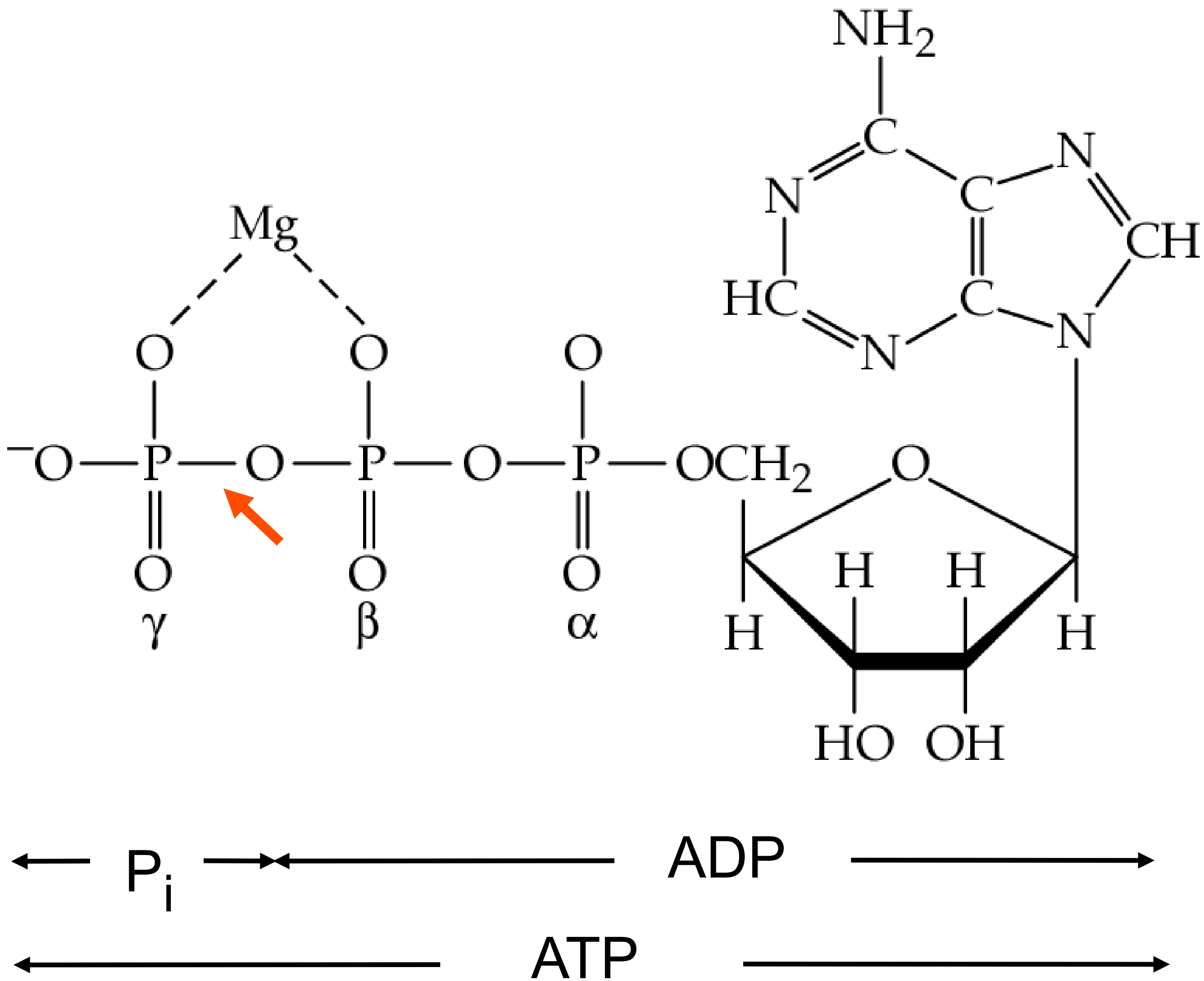
In vitro gliding assay (upside down assay)



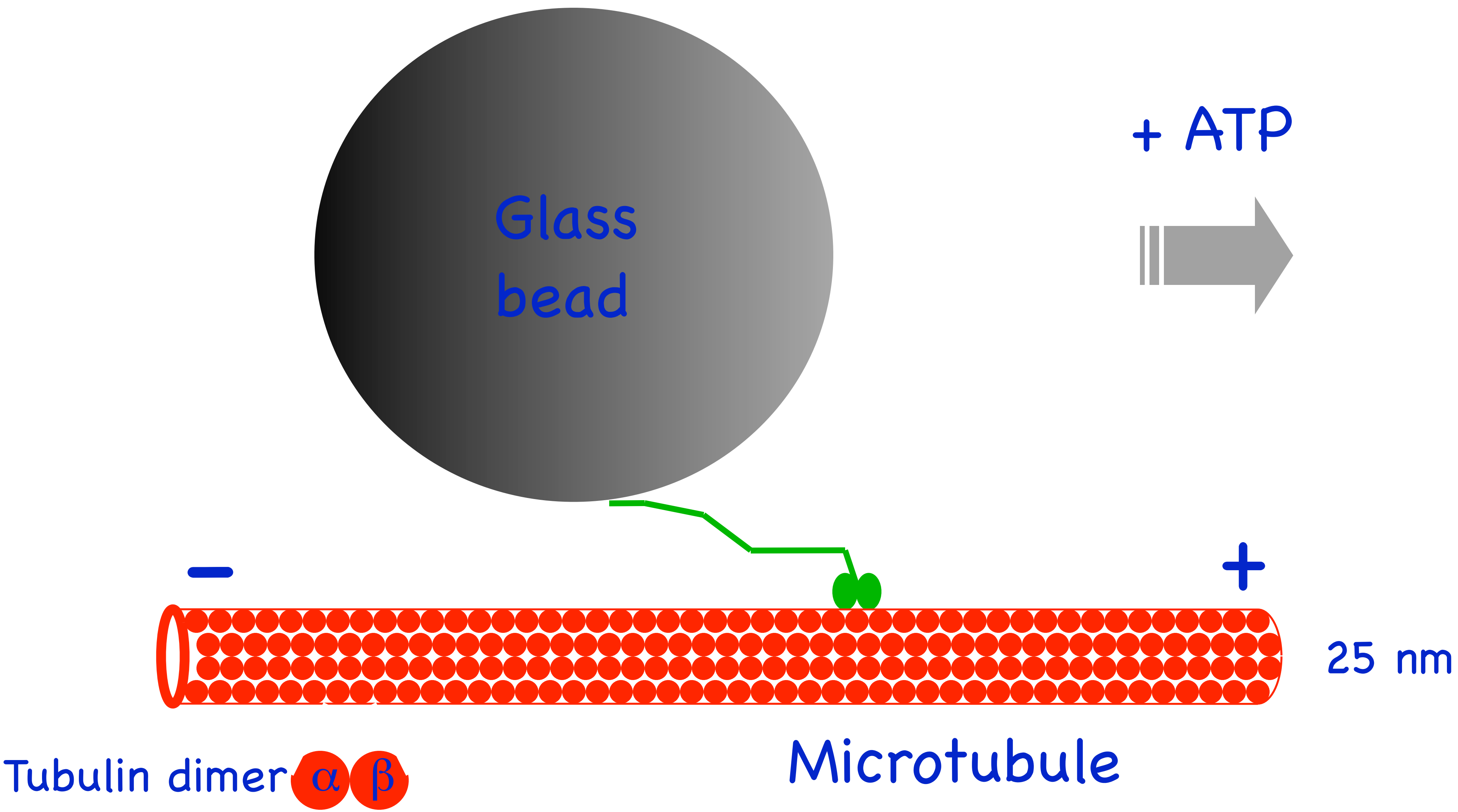
Microtubule gliding assay



ATP is required for motility

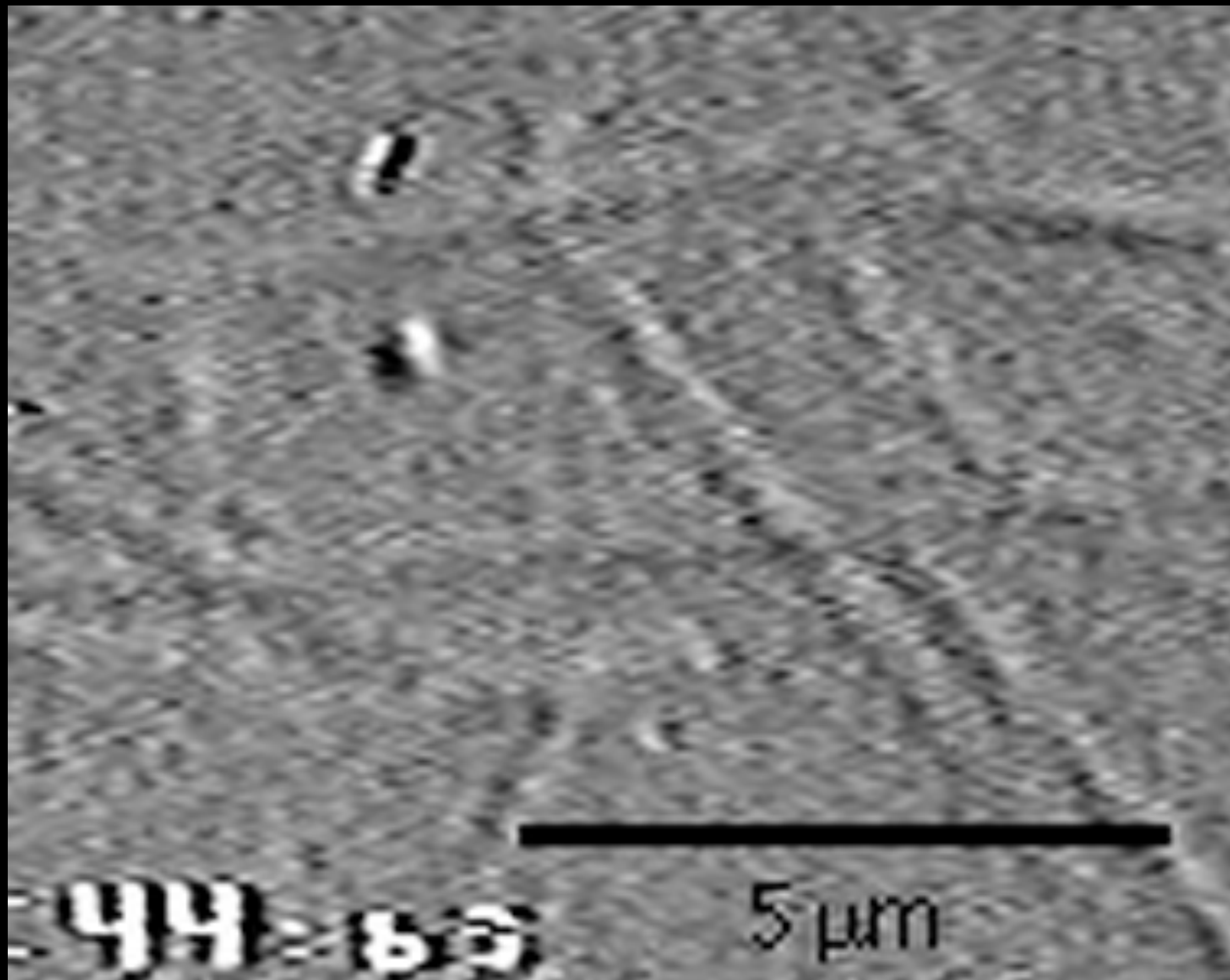


Bead Assay



Coy et al. J. Biol. Chem., 1999

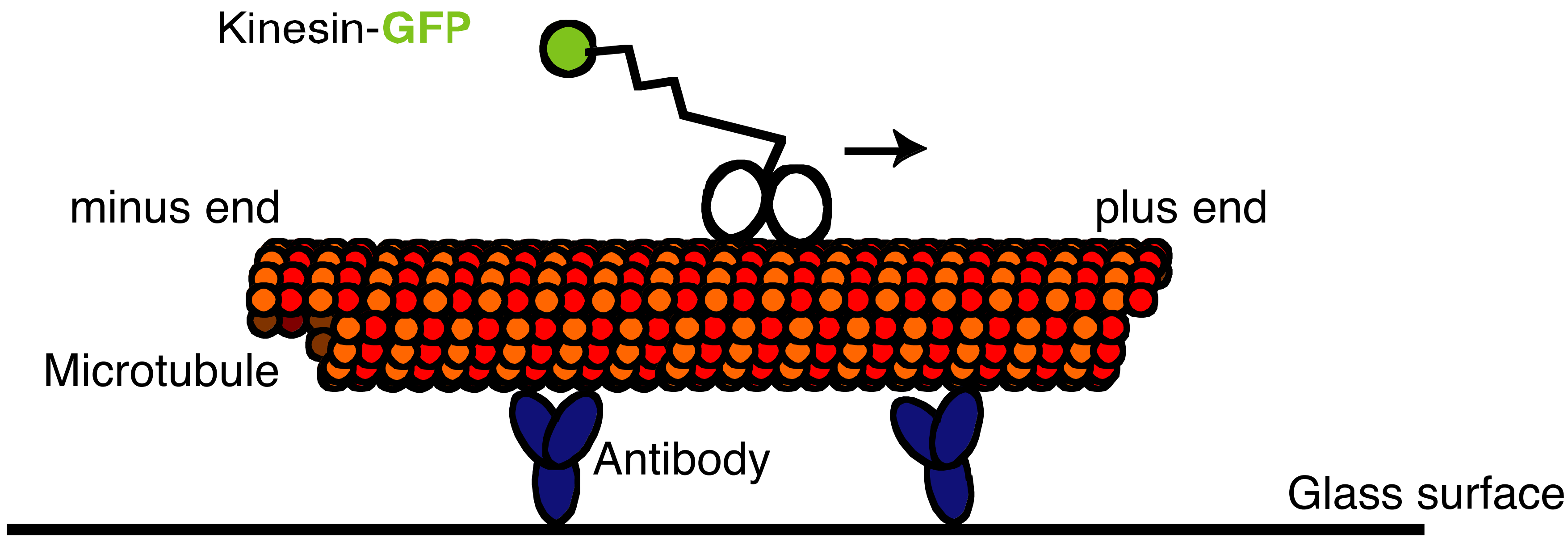
Bead Assay



real time

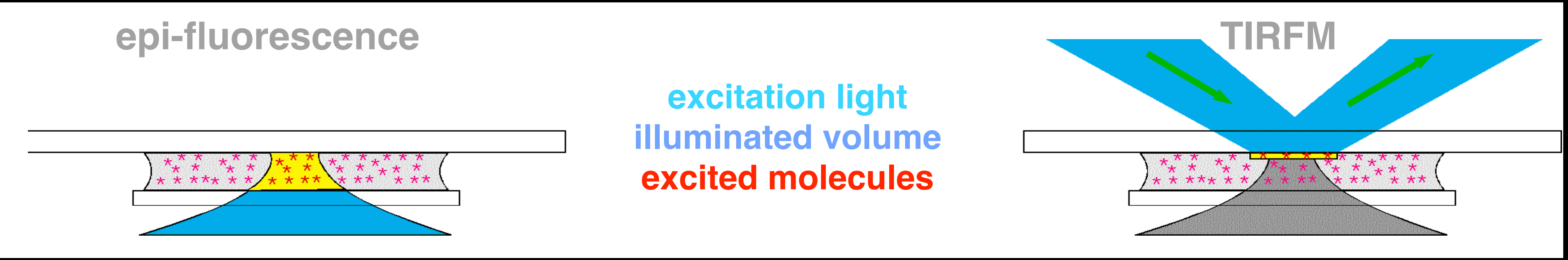
Coy et al. J. Biol. Chem., 1999

Kinesin is processive: single molecules move



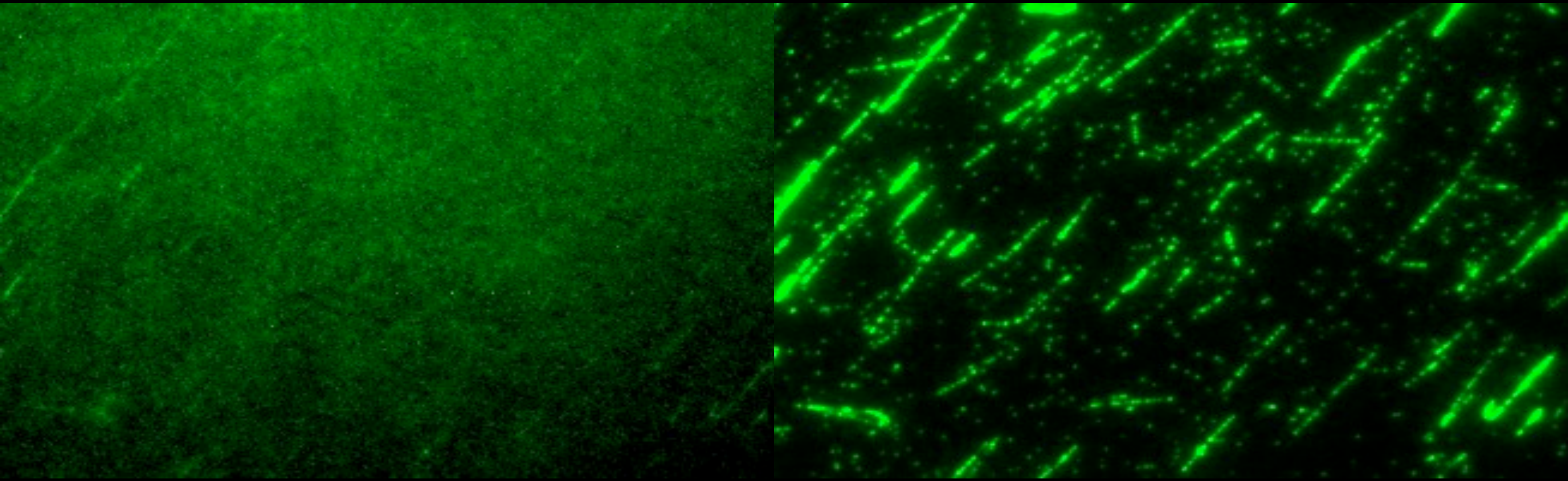
with Stefan Diez

Total-Internal-Reflection-Fluorescence (TIRF) microscopy



with Stefan Diez

Total-internal-reflection-fluorescence microscopy (TIRF)



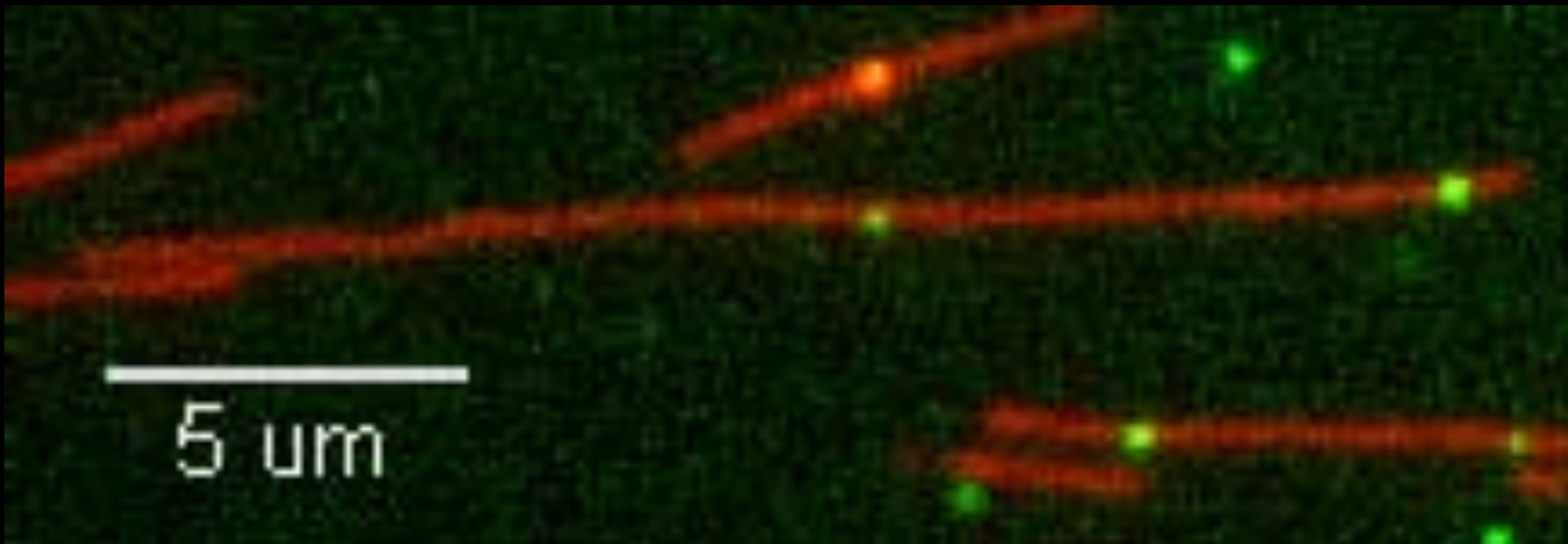
Fluorescence TIRF

Imaging area – 75 µm x 55 µm

GFP-MCAK on microtubules

with Stefan Diez

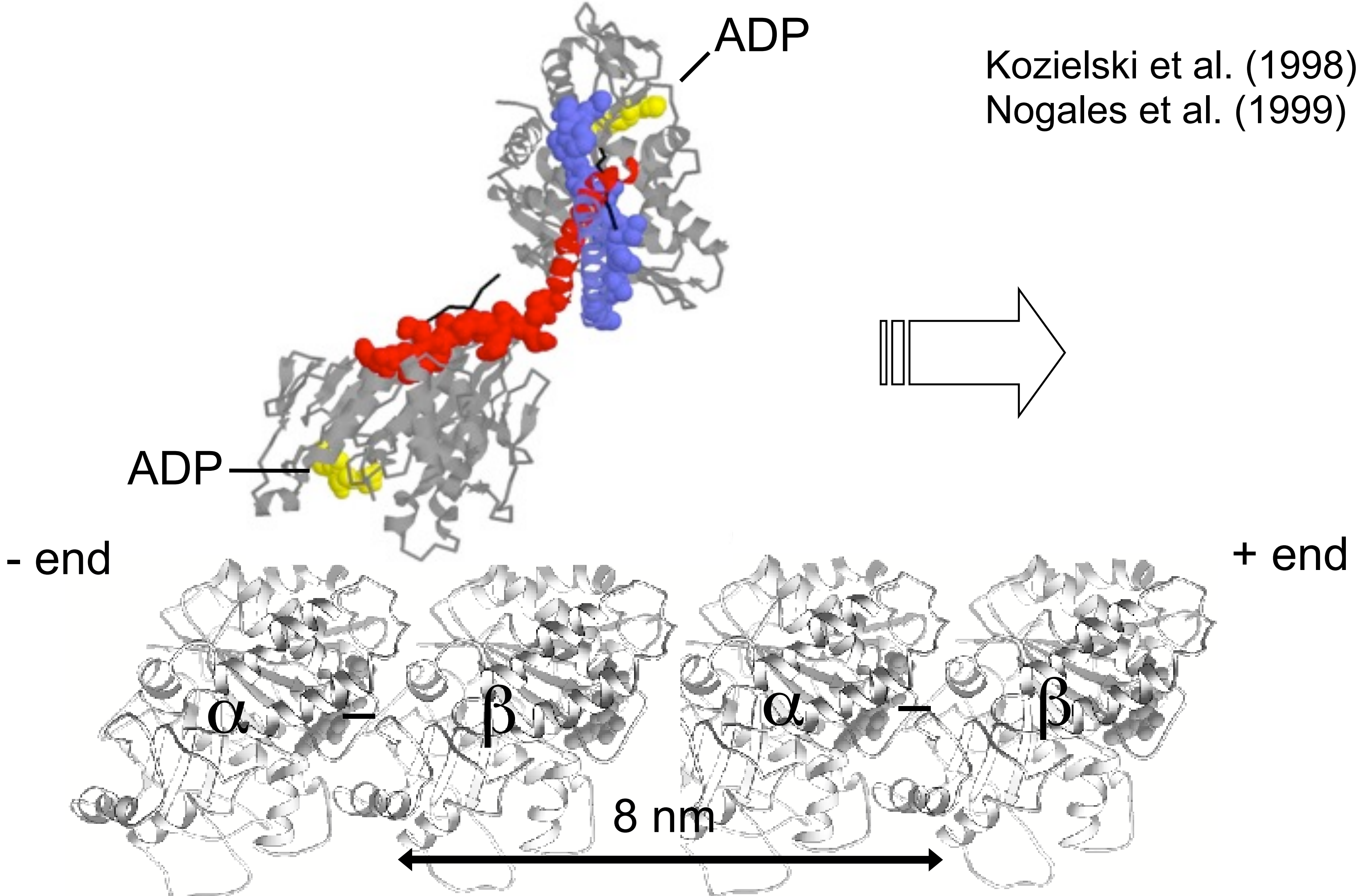
Processive motility of kinesin



with Stefan Diez

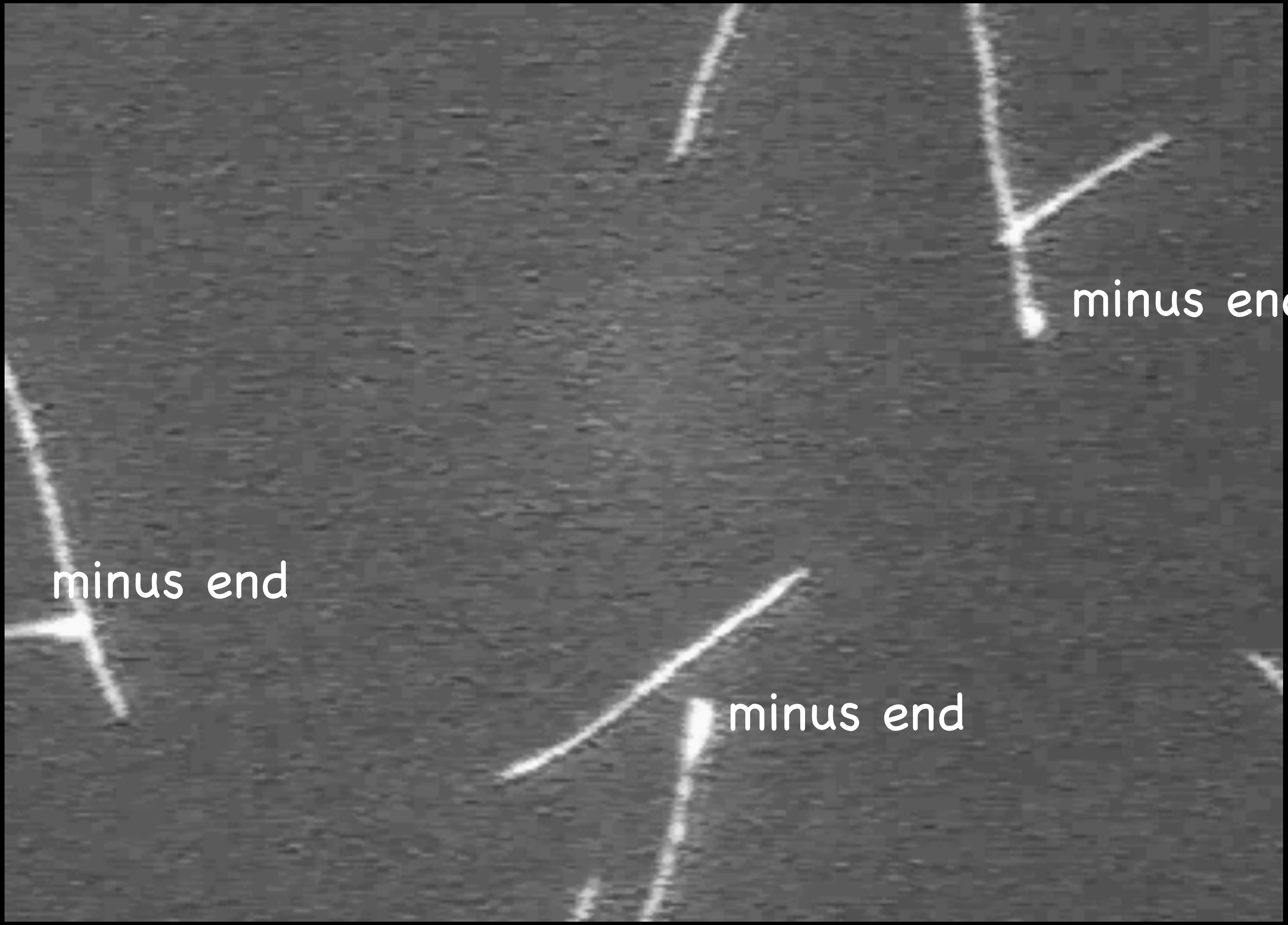
How does kinesin move?

Kozielski et al. (1998)
Nogales et al. (1999)



In which direction does kinesin move?

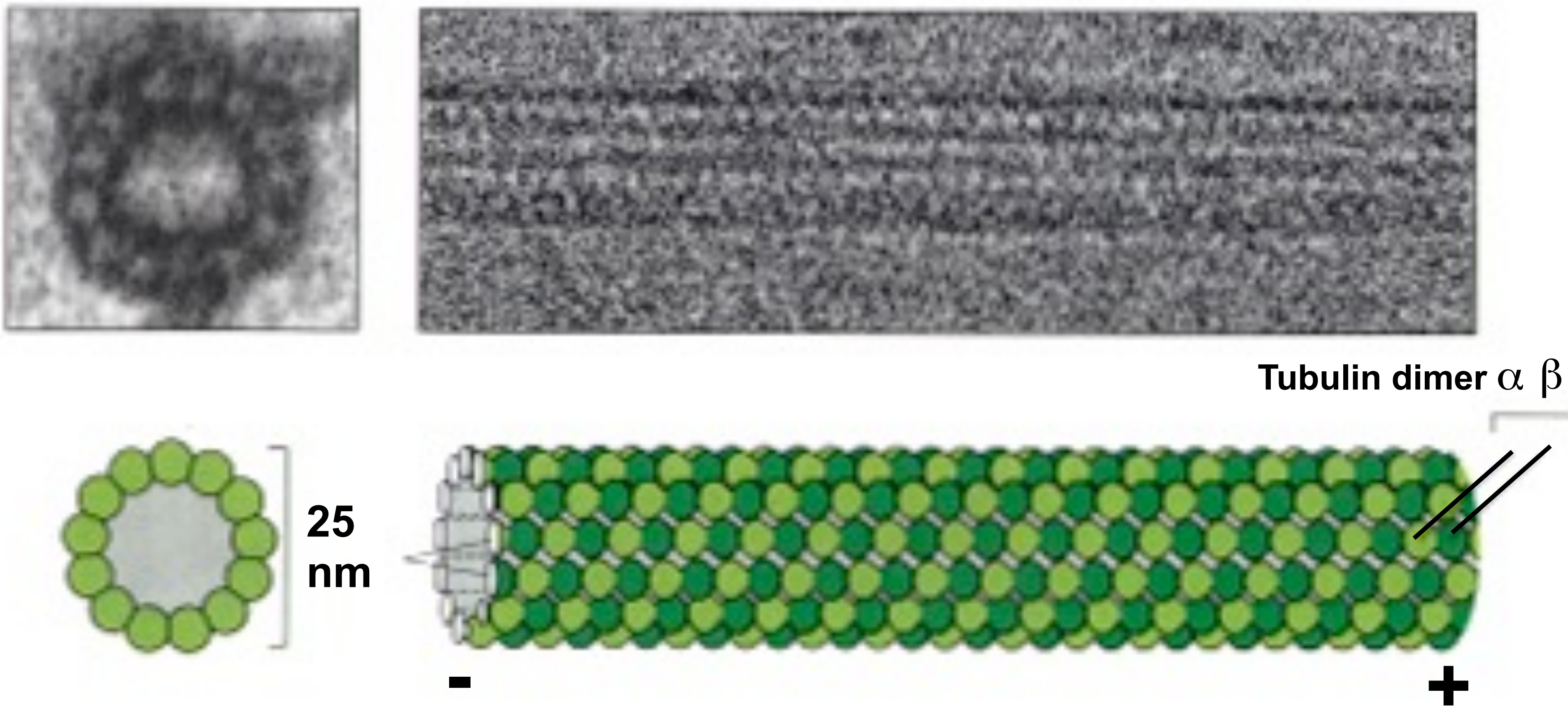
Kinesin moves towards the plus-end of the microtubule



Howard & Hyman 1993

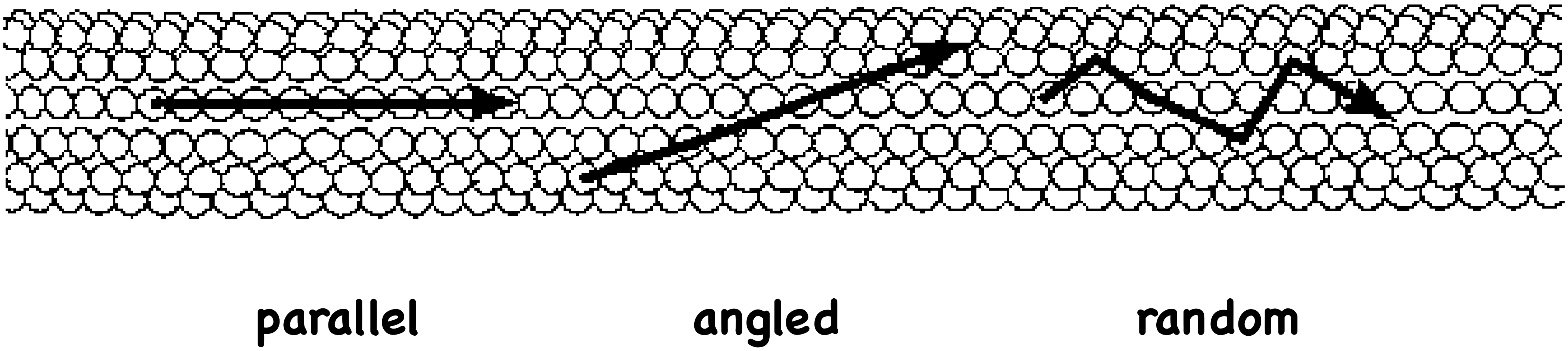
What path does kinesin follow on the microtubule surface?

Lattice structure of the microtubule

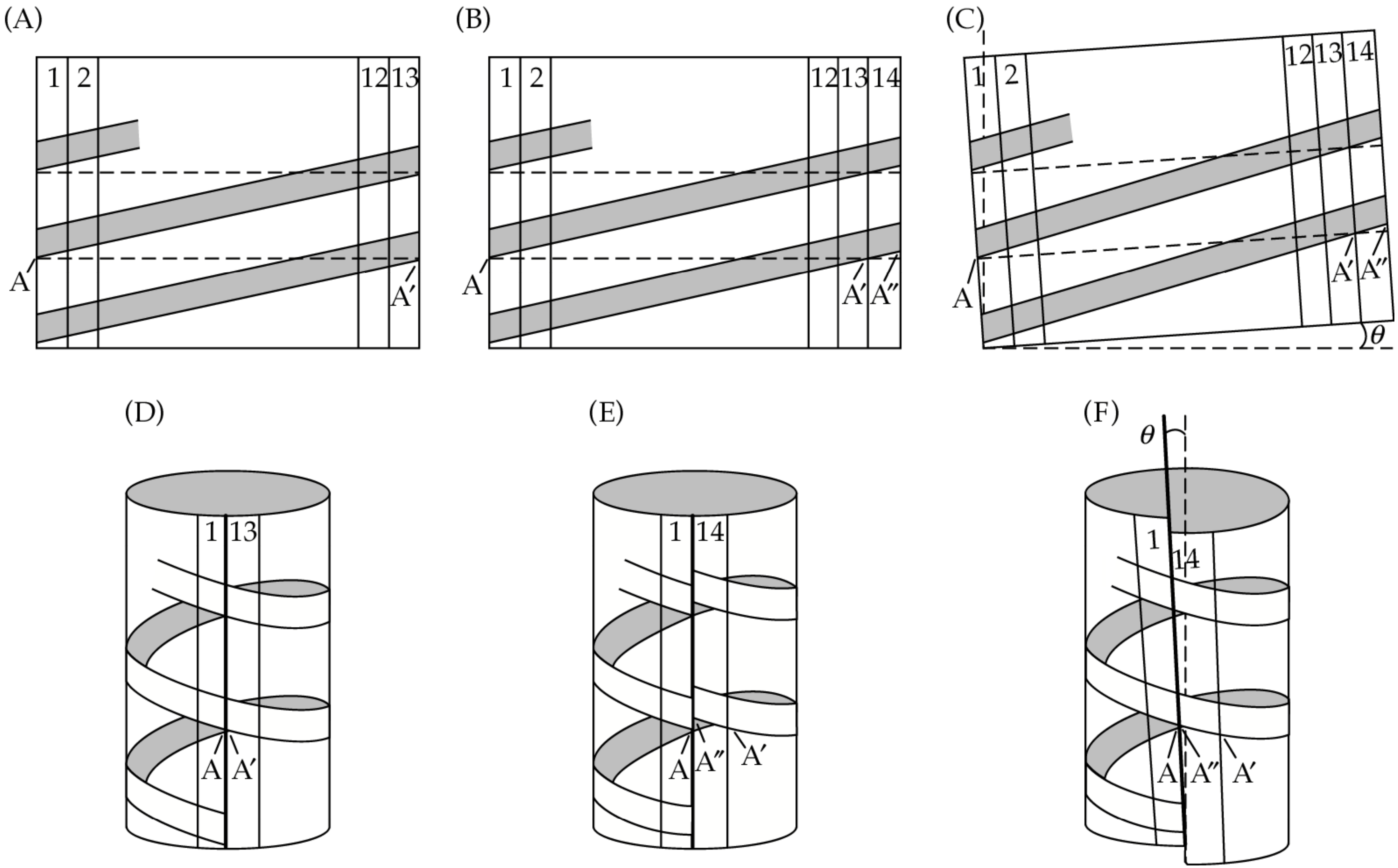


Alberts et al., Molecular Biology of the Cell

Which path does kinesin follow?

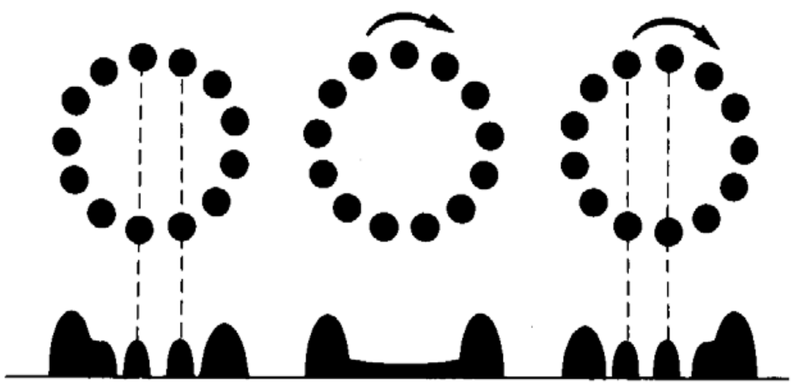


Lattice rotation model

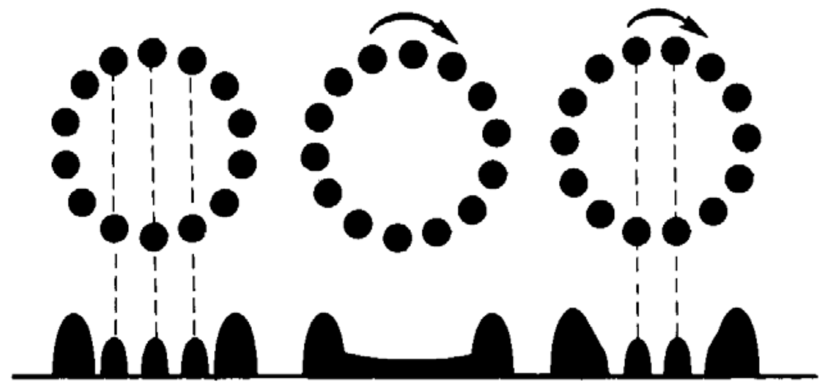


Dick Wade, Grenoble

Moire pattern reveals the supertwist



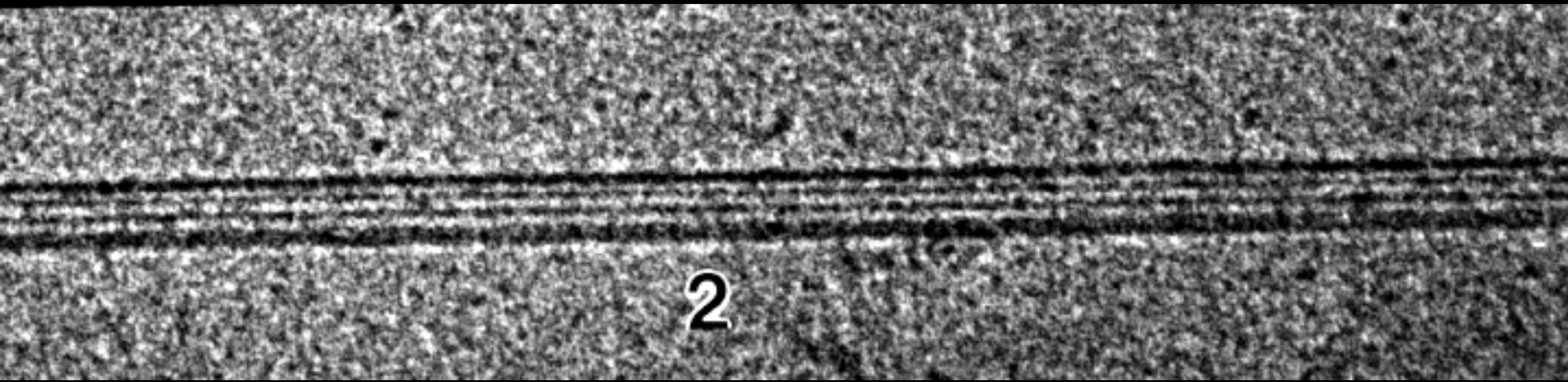
13 protofilaments



14 protofilaments

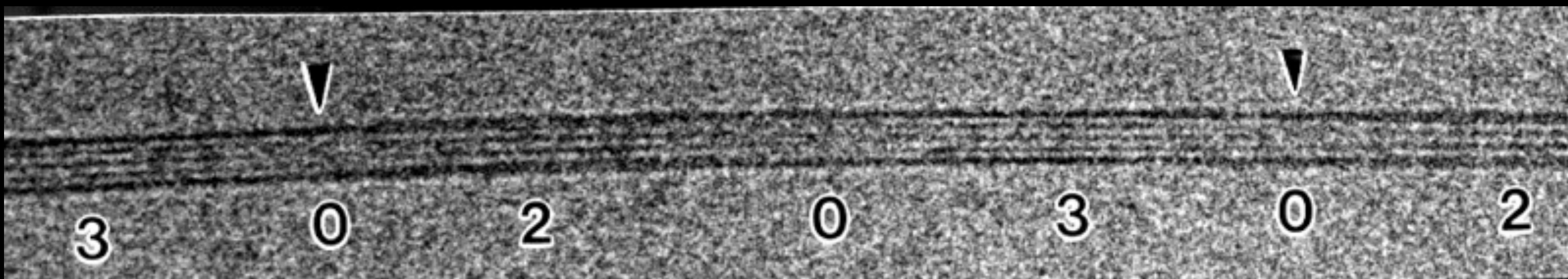
Dick Wade, Grenoble

13 Protofilaments



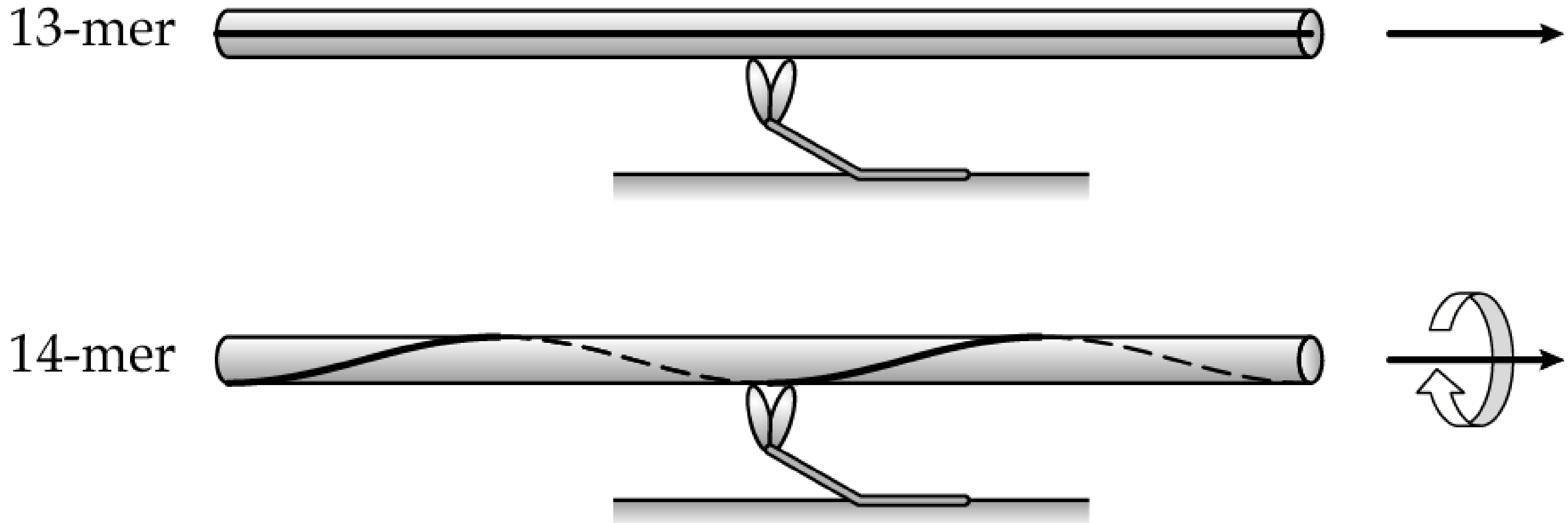
Ray et al., J. Cell Biol. 1993

14 Protofilaments



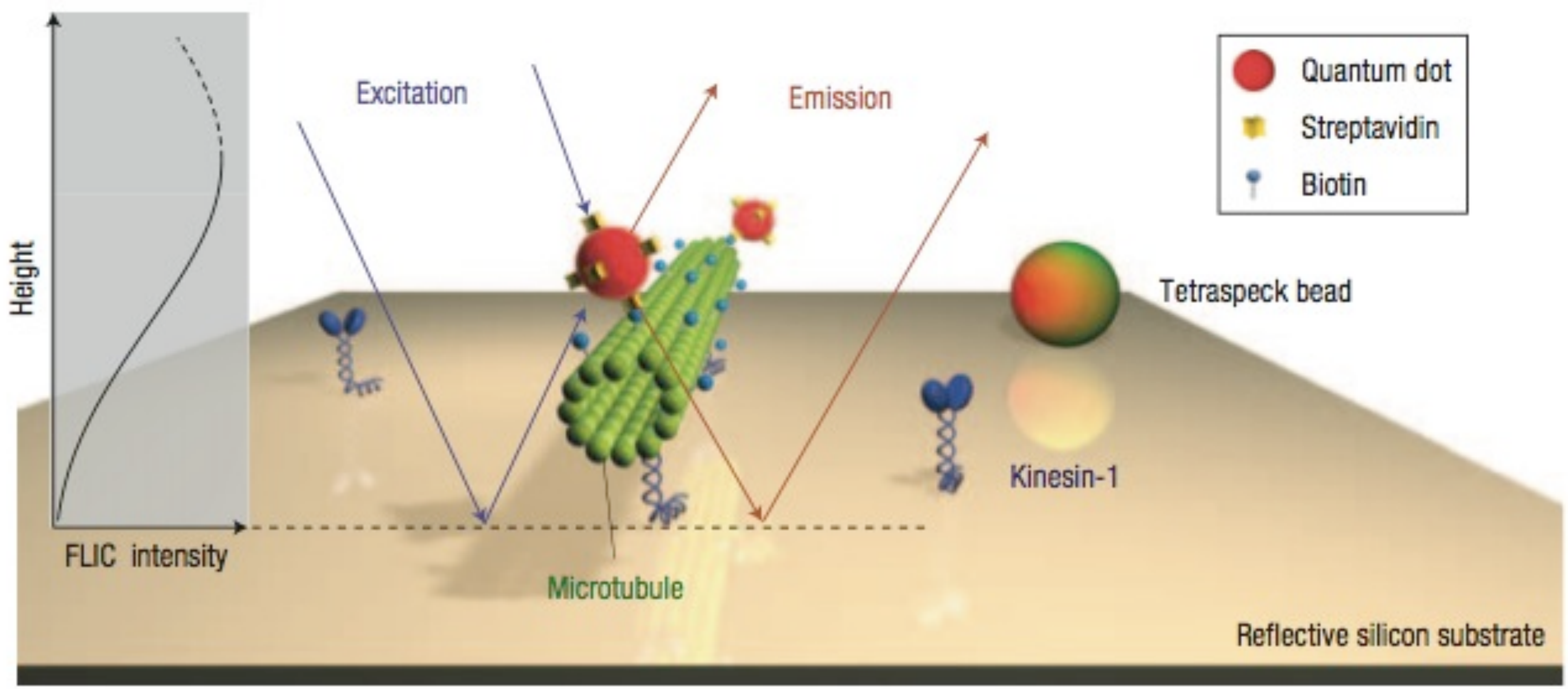
Ray et al., J. Cell Biol. 1993

Rotation of supertwisted microtubules



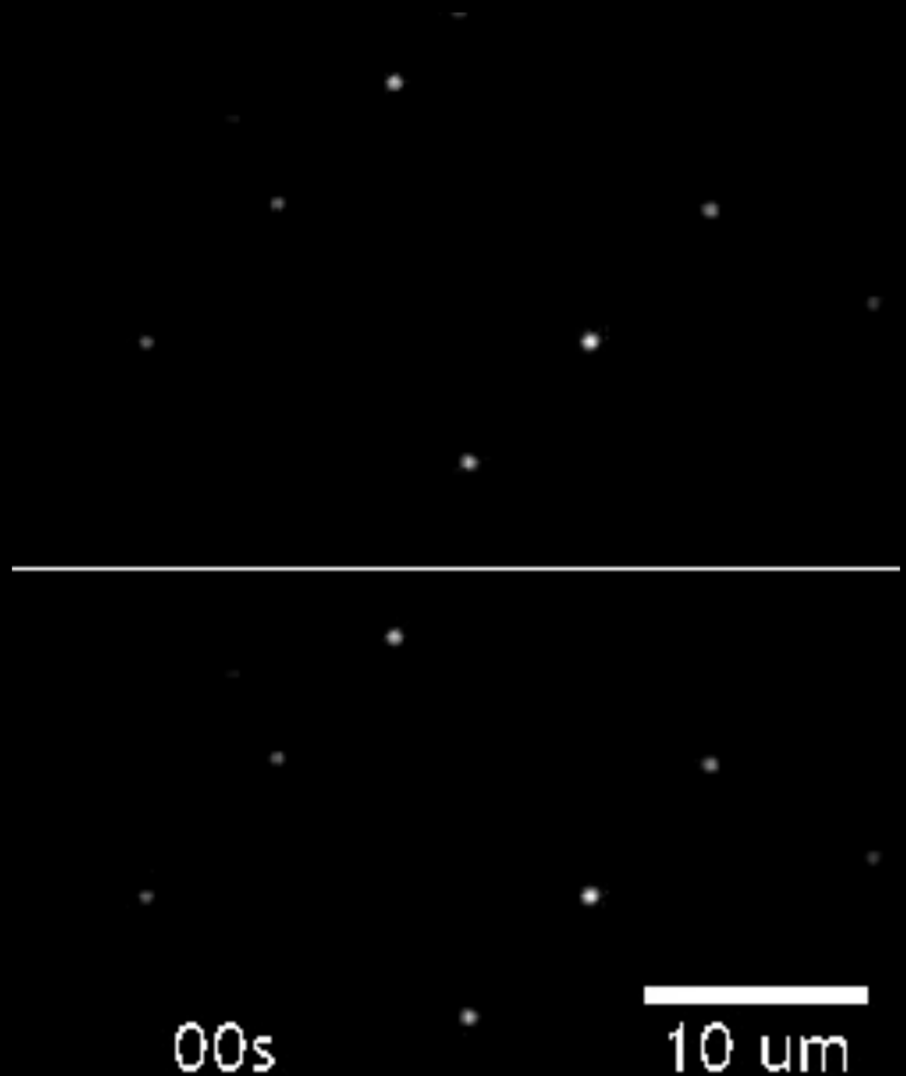
Ray et al., J. Cell Biol. 1993

Fluorescence interference contrast (FLIC) microscopy



Nitzsche et al. Nature Nanotech. (2008)
(**Stefan Diez**, Dresden)

Rotation of 14-protofilament microtubules

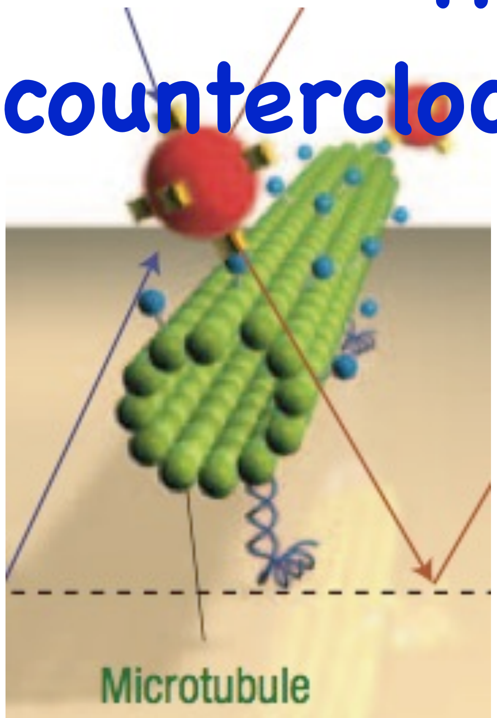


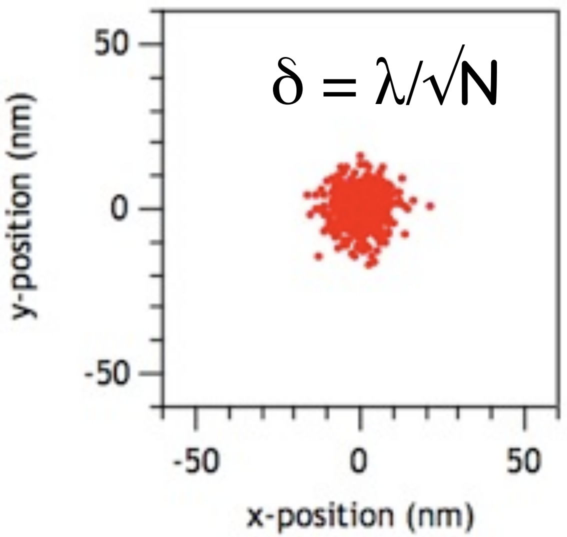
Video

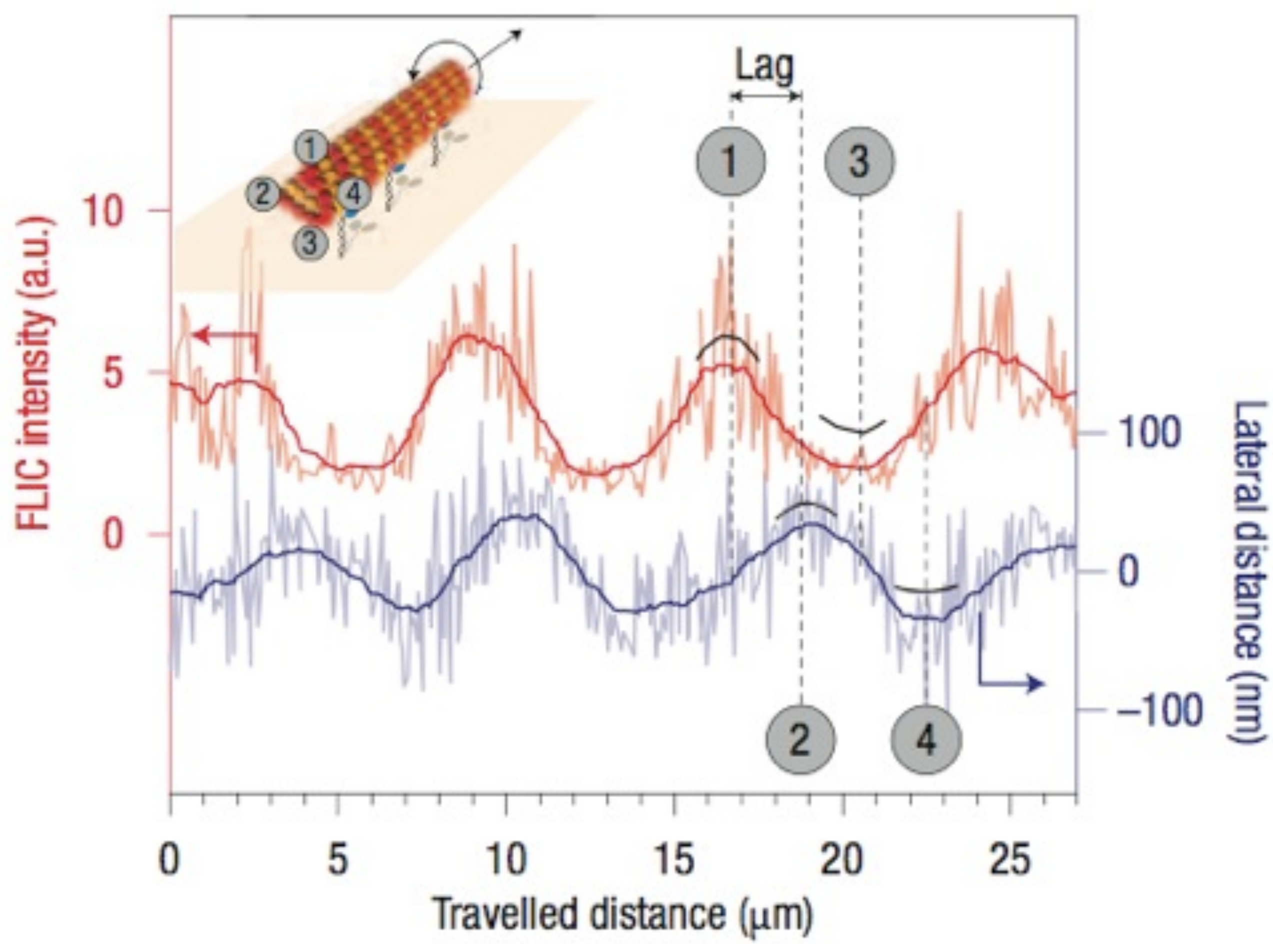
Maximum projection

Nitzsche et al. Nature Nanotech. (2008)

Handedness of rotation
counterclockwise → along the protofilament

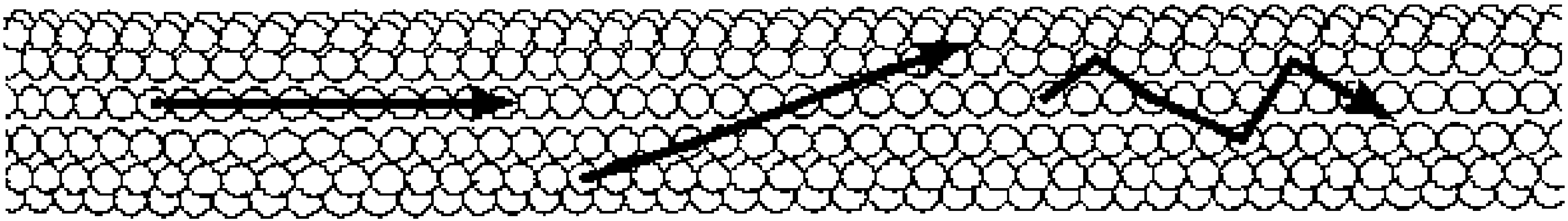






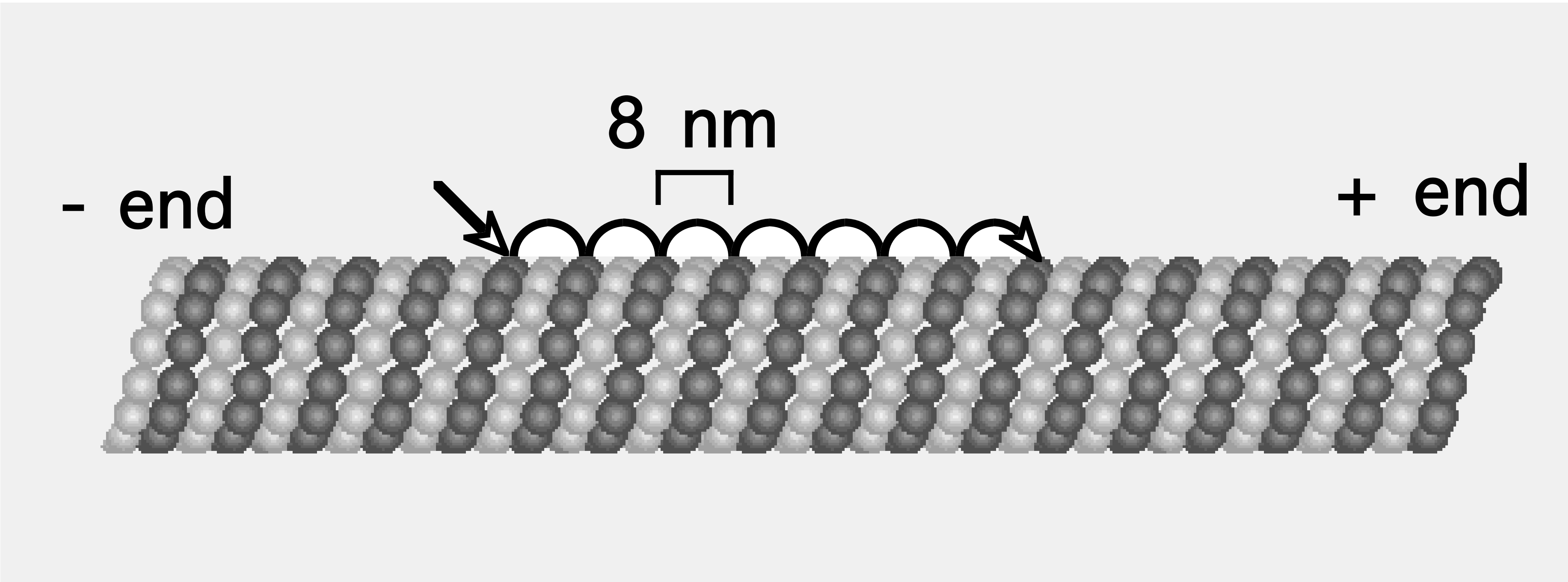
Nitzsche et al. Nature Nanotech. (2008)

Kinesin follows the protofilament axis

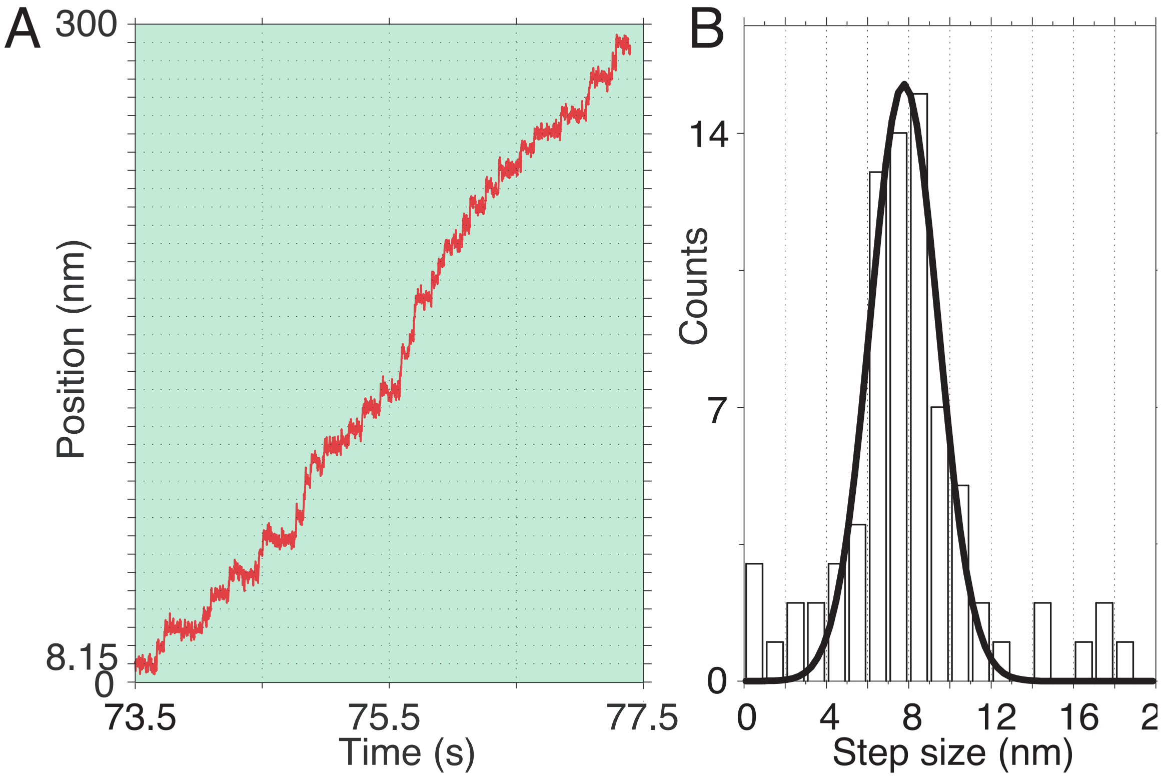


parallel	angled	random
YES	NO	NO

Kinesin's path on the microtubule



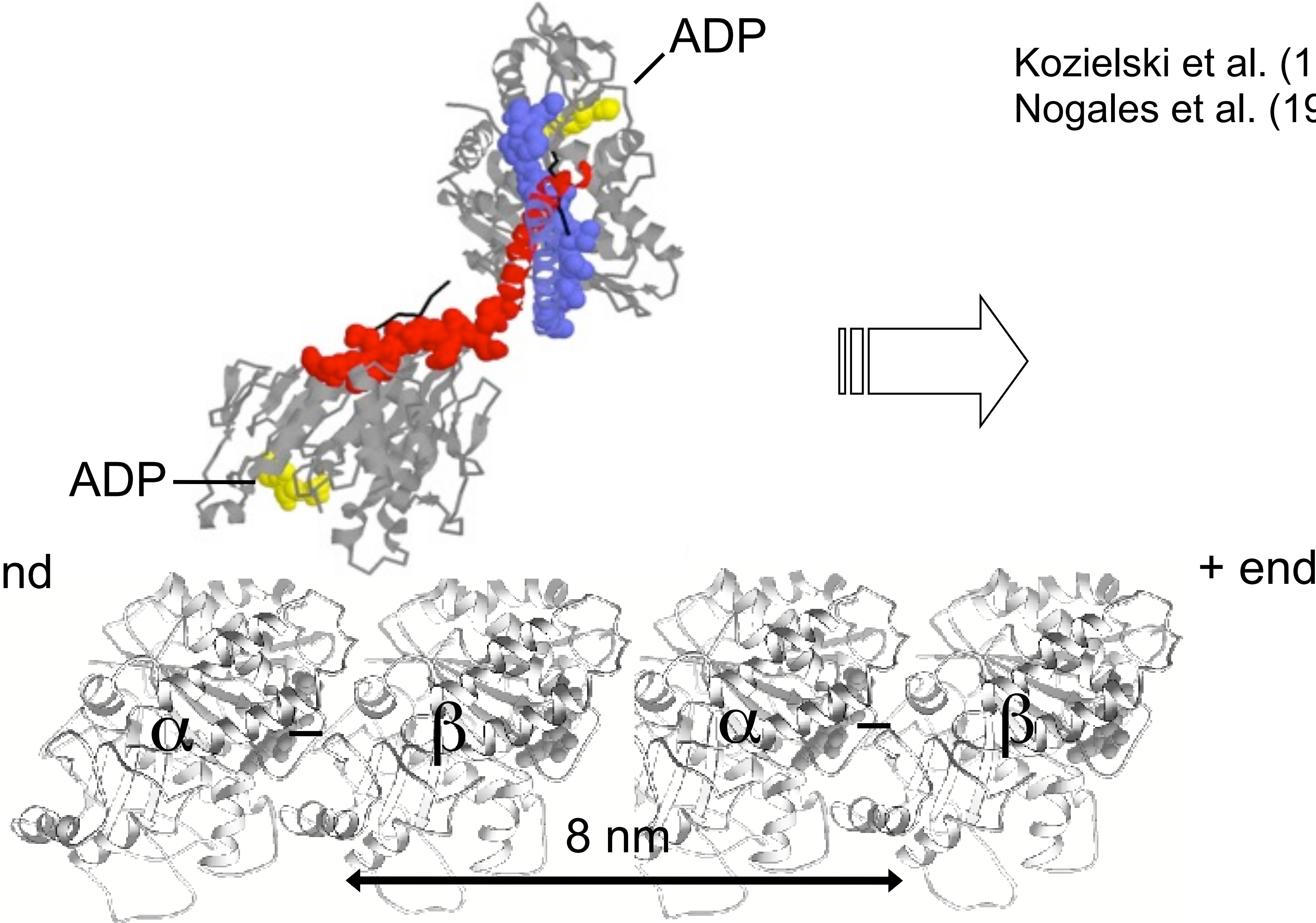
Direct measurement of 8-nm steps



Volker Bormuth

How does kinesin get to the next binding site?

Kozielski et al. (1998)
Nogales et al. (1999)



Outline

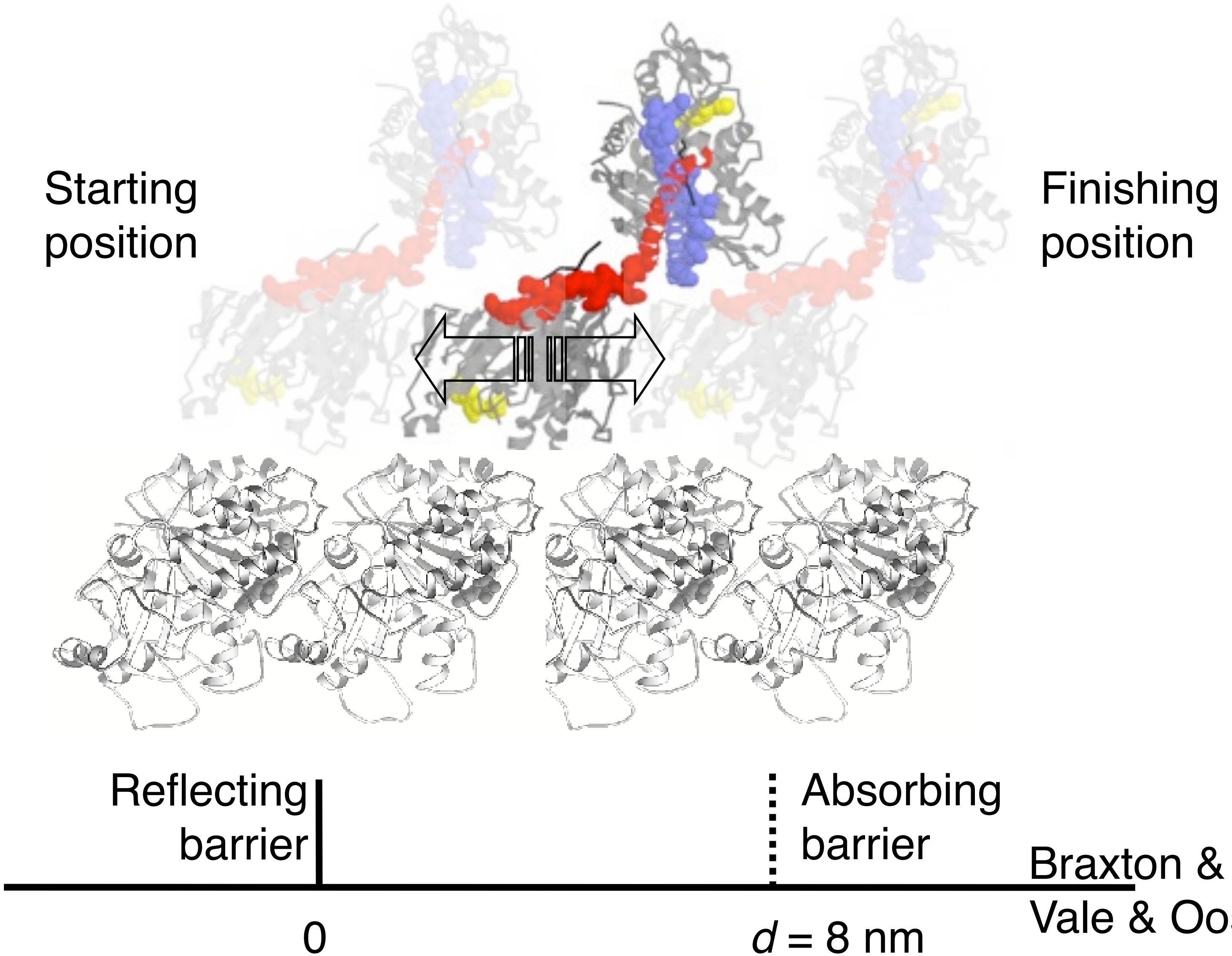
1. Single-molecule techniques can be used to study movement of purified motor proteins

2. Role of **fluctuations** in the motor reaction

3. Protein friction limits motor speed and efficiency

4. Force gating of motor proteins: active mechanical circuits underlying cell motility

“Ratchet diffusion” model



Braxton & Yount 1989
Vale & Oosawa 1990

Force generated by the "Ratchet diffusion" mechanism

Prediction:

First-passage time: $t = d^2/2D = d^2\gamma/2kT$

d = step size = 8 nm

γ = viscosity

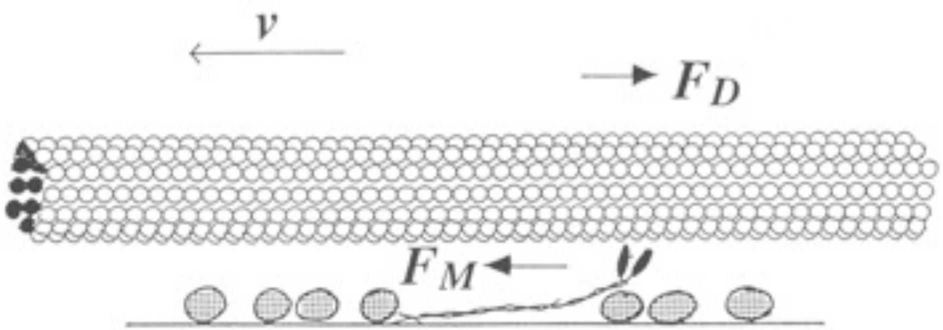
Maximum force against a viscous load:

$f_{drag} = \gamma v_{max} = \gamma d/t = 2kT/d = \boxed{1\ pN}$

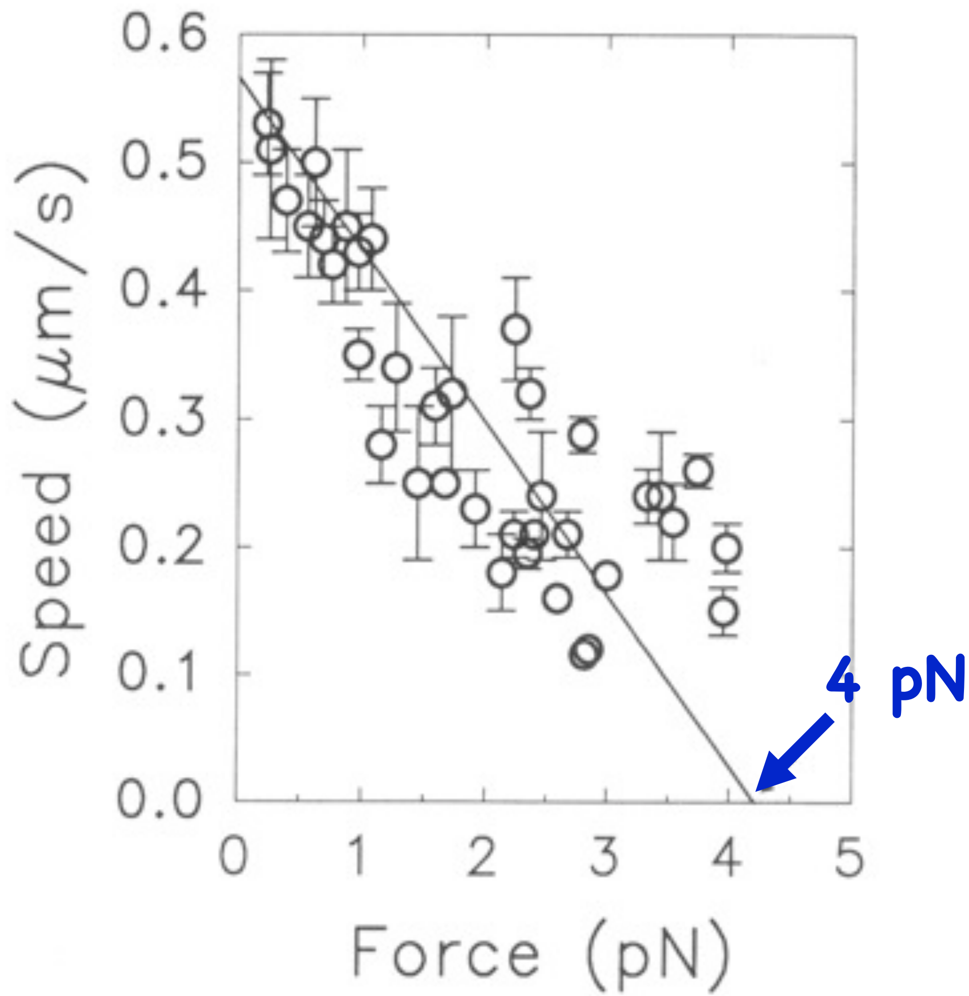
Hunt et al. (1994)

Single-kinesin force against a viscous load

increase viscosity 100-fold

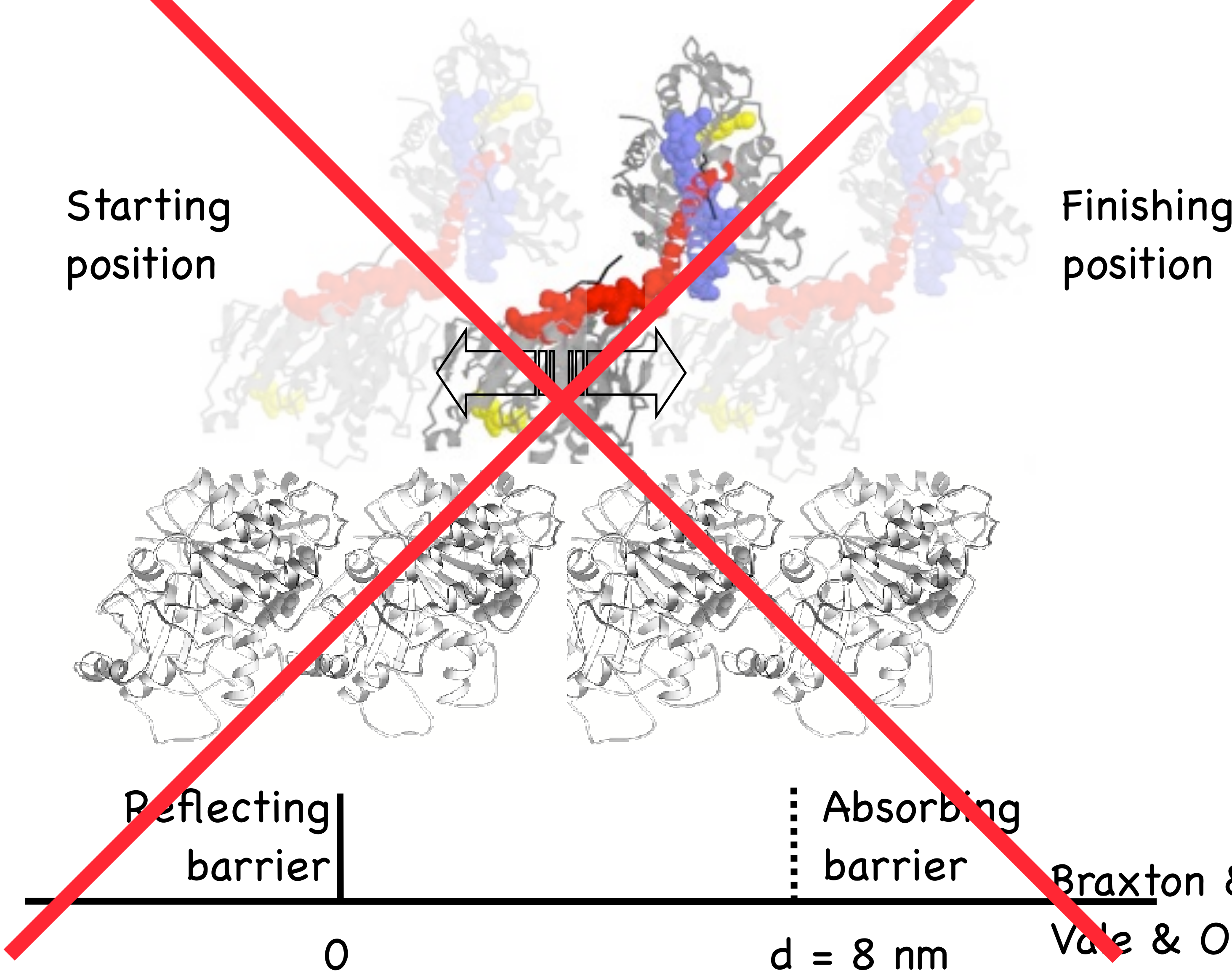


"upside-down assay"



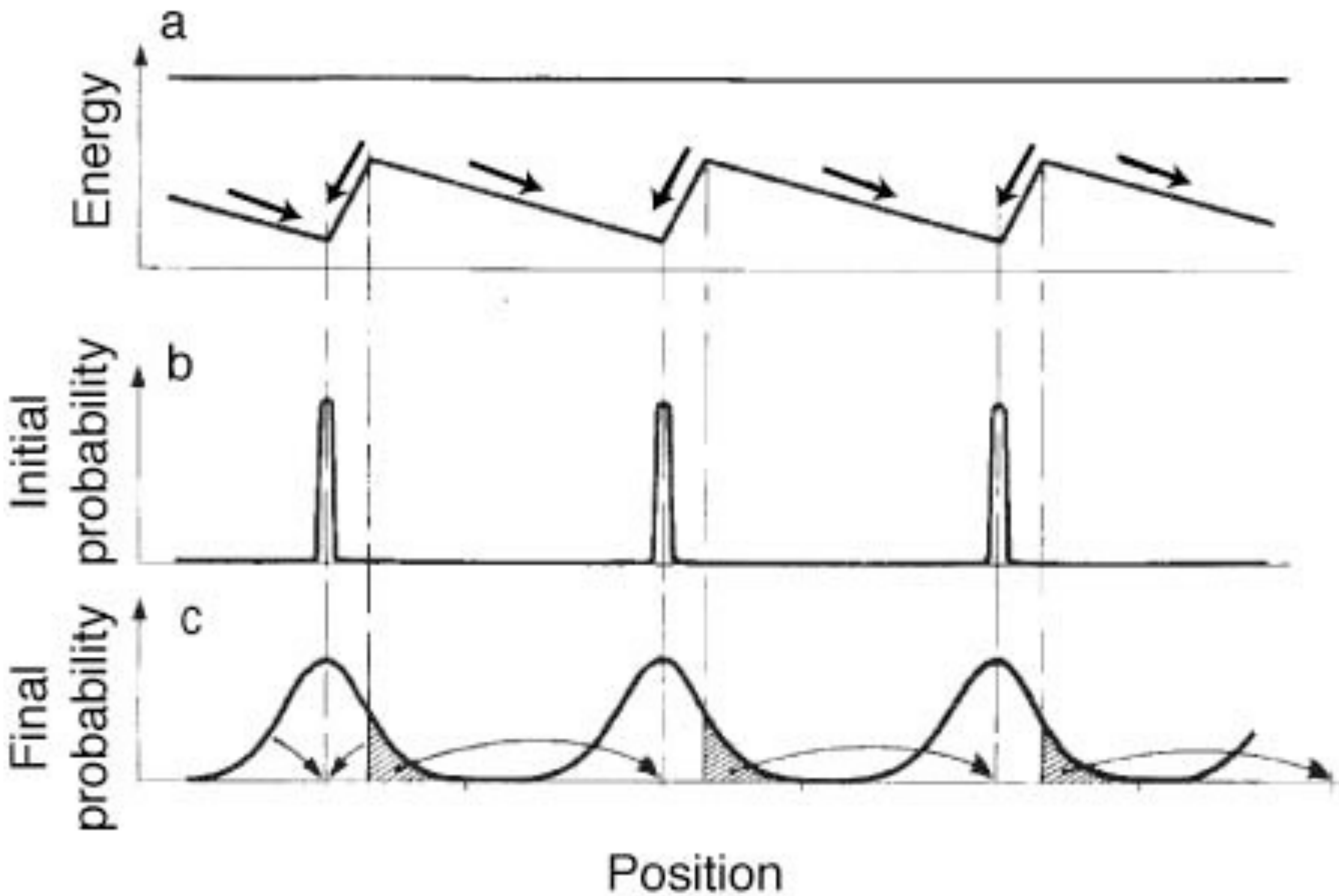
Hunt et al. (1994)

"Ratchet diffusion" model



Braxton & Yount 1989
Vale & Oosawa 1990

Time-varying (flashing) ratchets



Roussellet et al. 1994
and see also Astumian & Bier 1994

Time-varying (flashing) ratchets

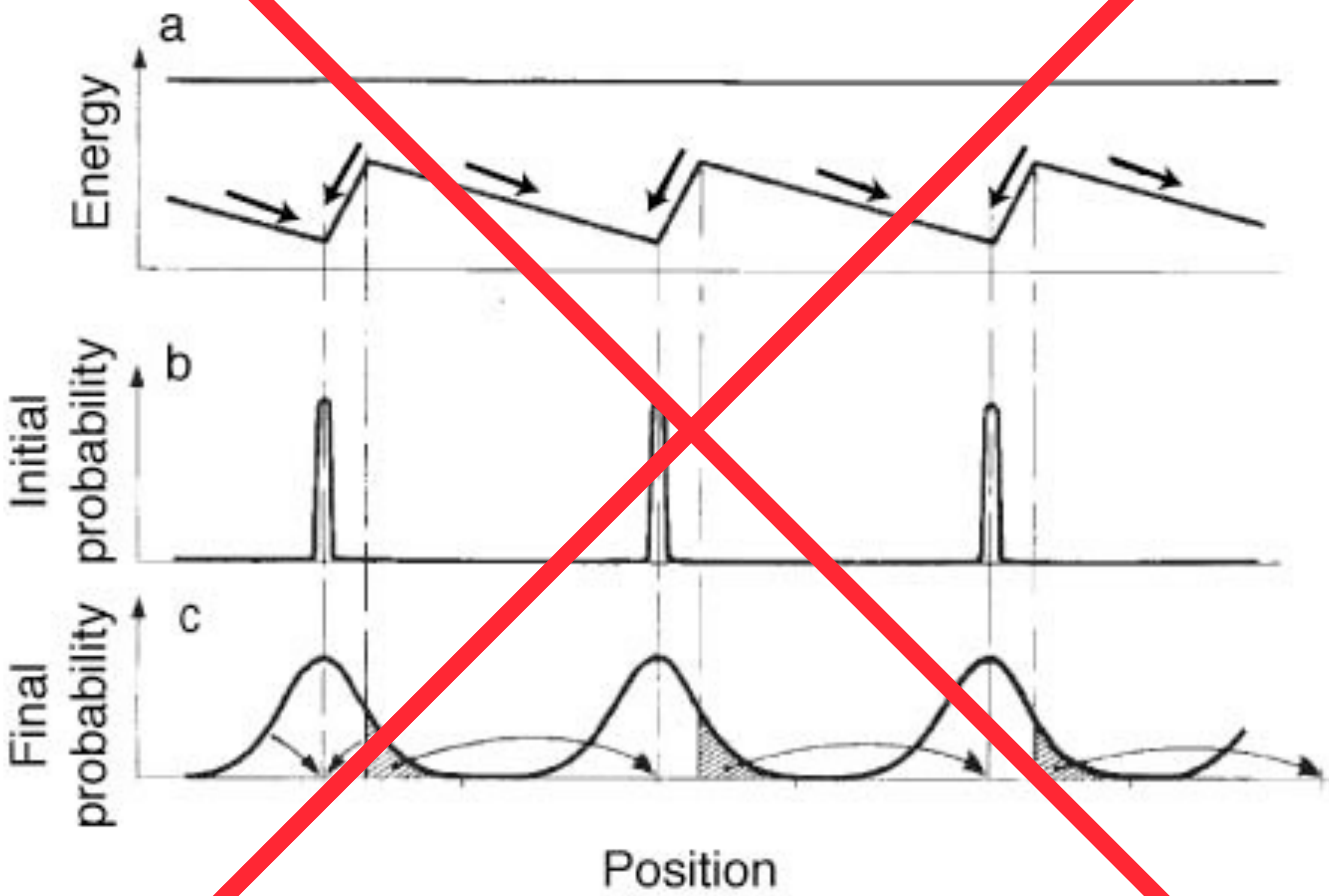
Prediction:

Because there is only a <50% chance of progressing to the next site (>50% chance of staying put or going backwards) then it takes at least 2 ATP on average to move 8 nm

Experiment:

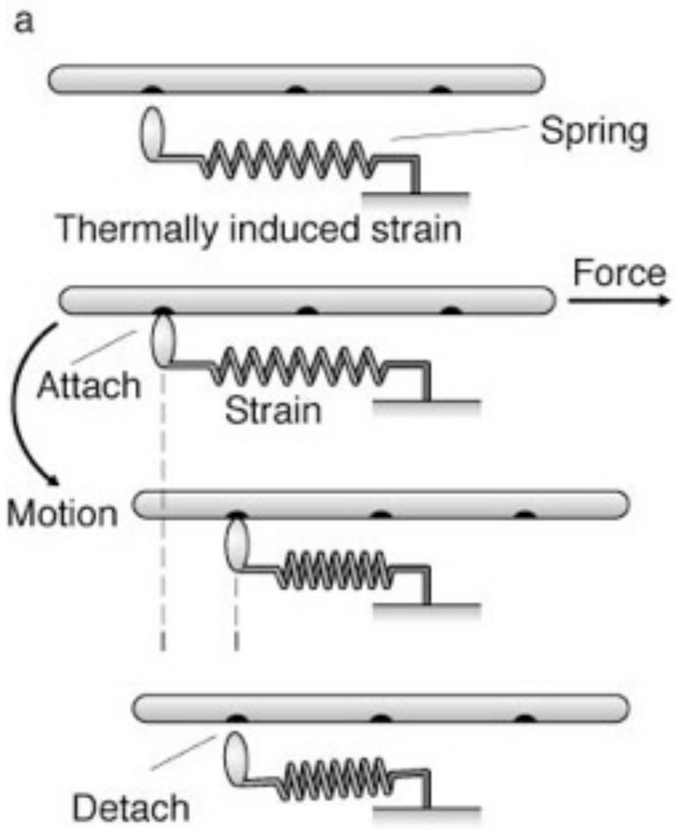
One ATP hydrolyzed per step (Coy et al. J. Biol. Chem. 1999)

Time-varying (flashing) ratchets



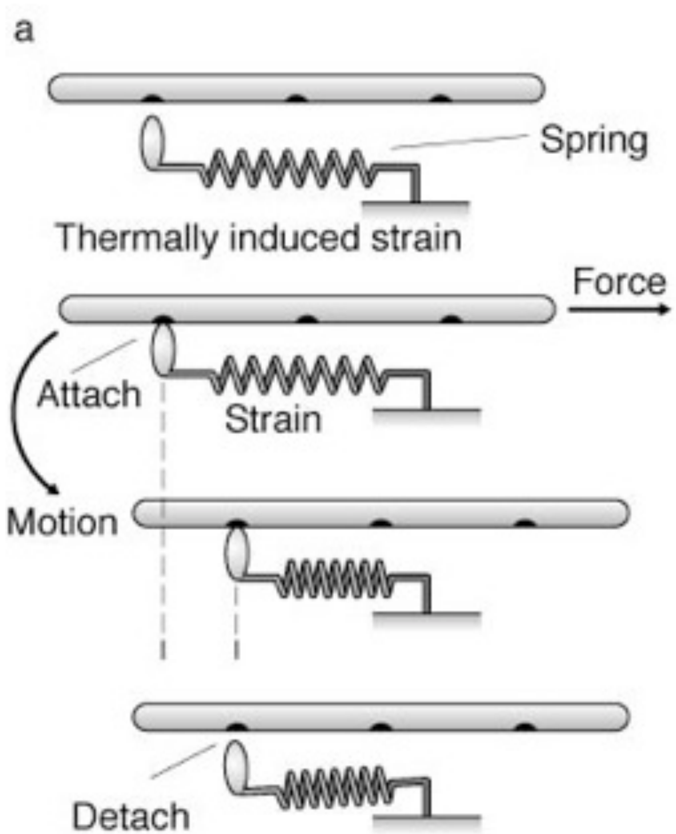
Roussellet et al. 1994
and see also Astumian & Bier 1994

Thermal ratchet model



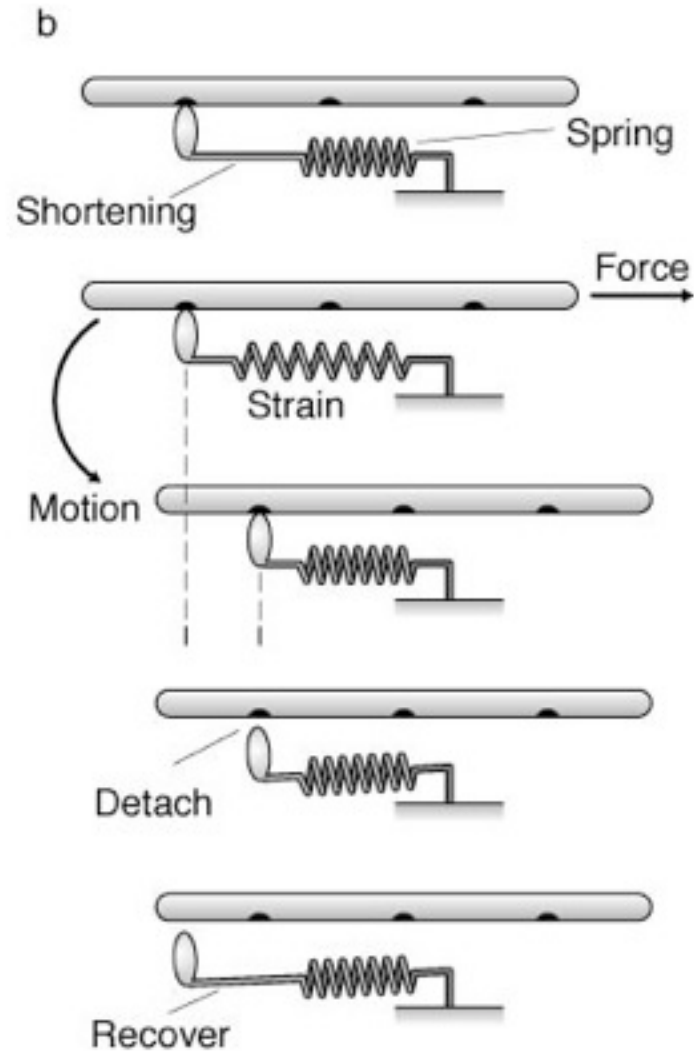
Huxley 1957
Cordova et al. 1992

Thermal ratchet model



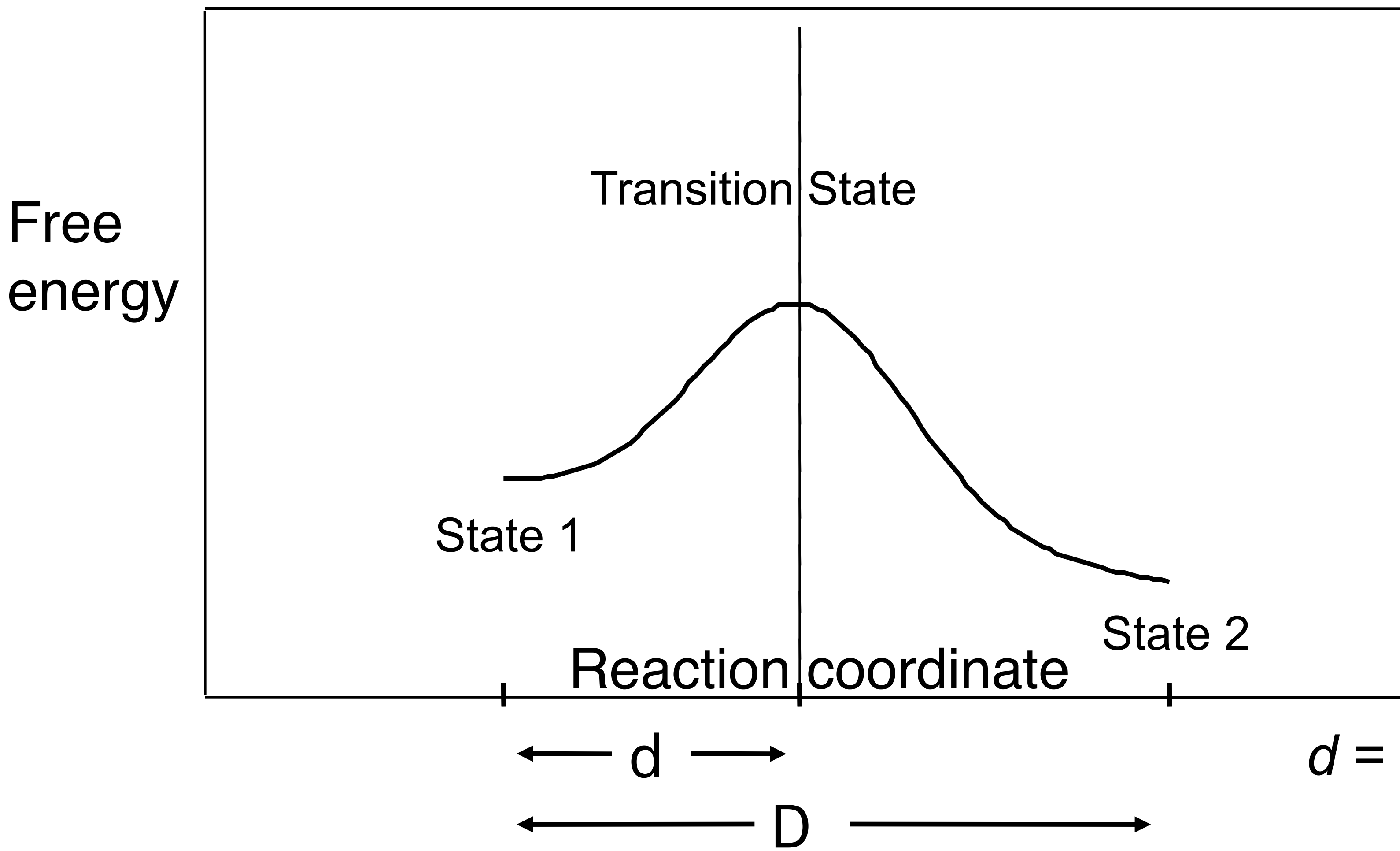
Huxley 1957
Cordova et al. 1992

Powerstroke model



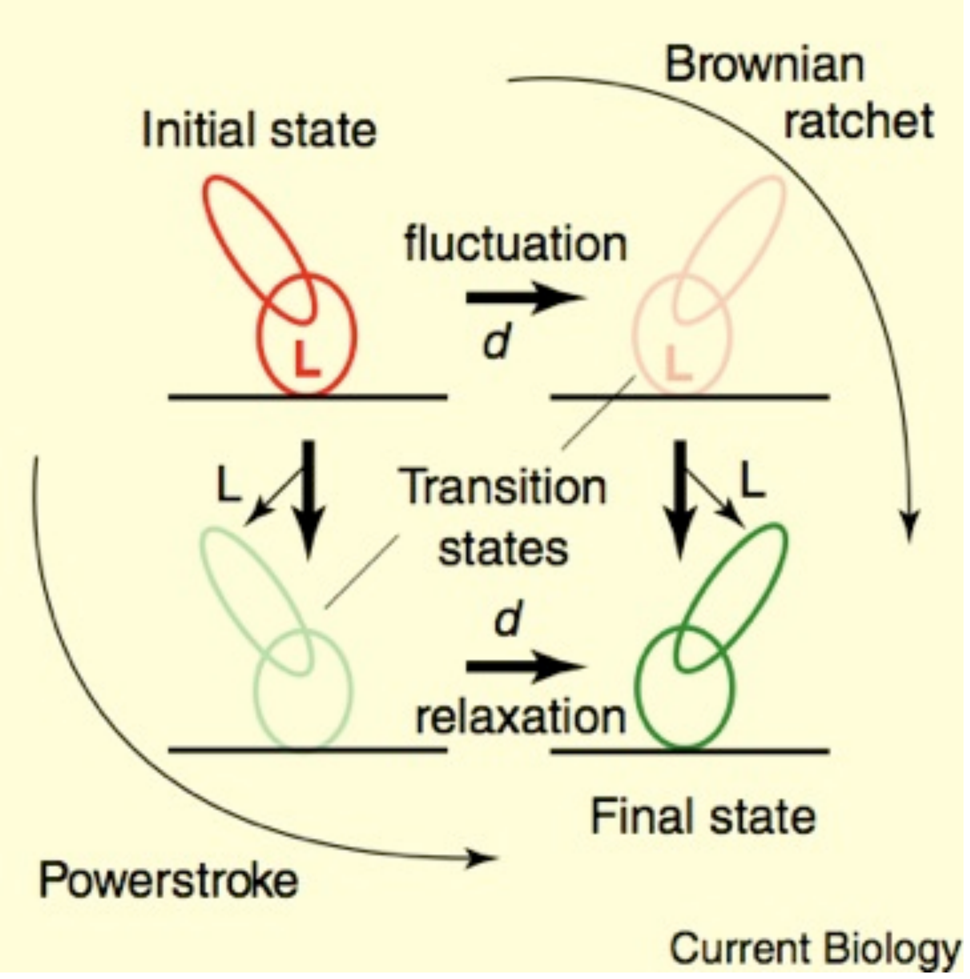
Eiesenberg & Hill 1978
Parmeggiani et al. 1999

Transition state concept



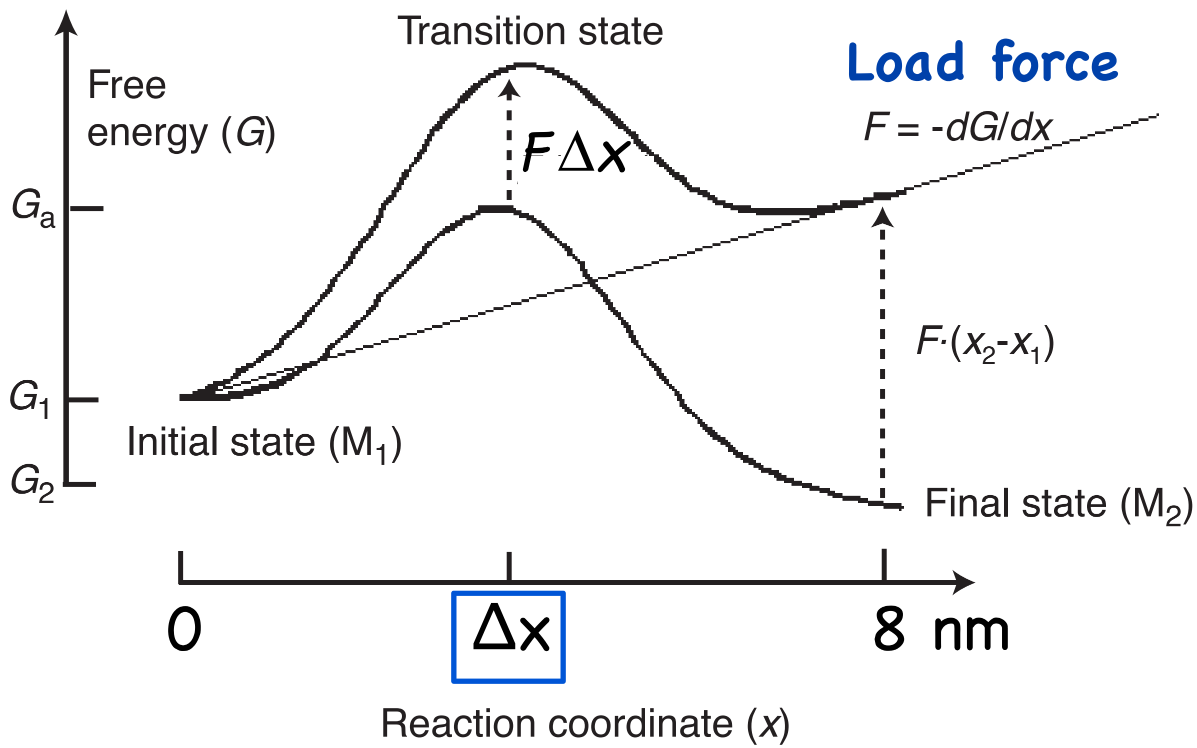
$d = f{\cdot}D$

What is the position of the transition state?



Howard, Curr. Biol. 2006

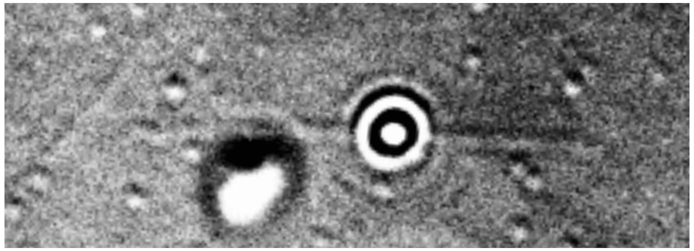
Force can be used to measure the position of the transition state



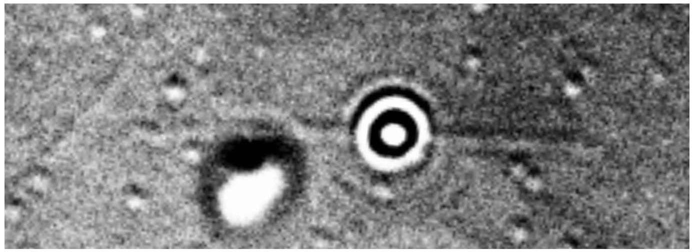
$$k_+ = k_0 \exp\left(-\frac{F \cdot \Delta x}{kT}\right)$$

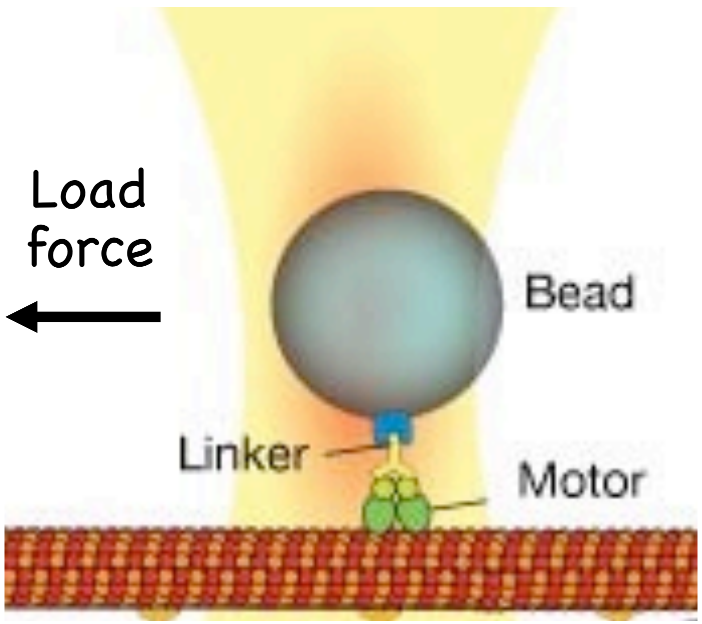
(Kramers 1940)

Force-velocity curve (kinesin)

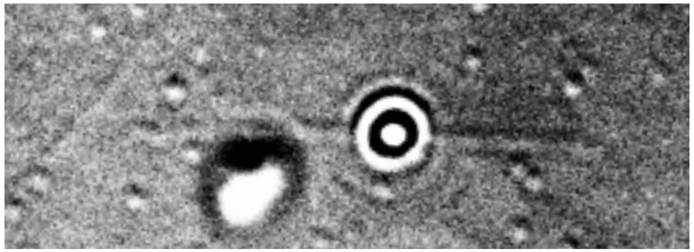


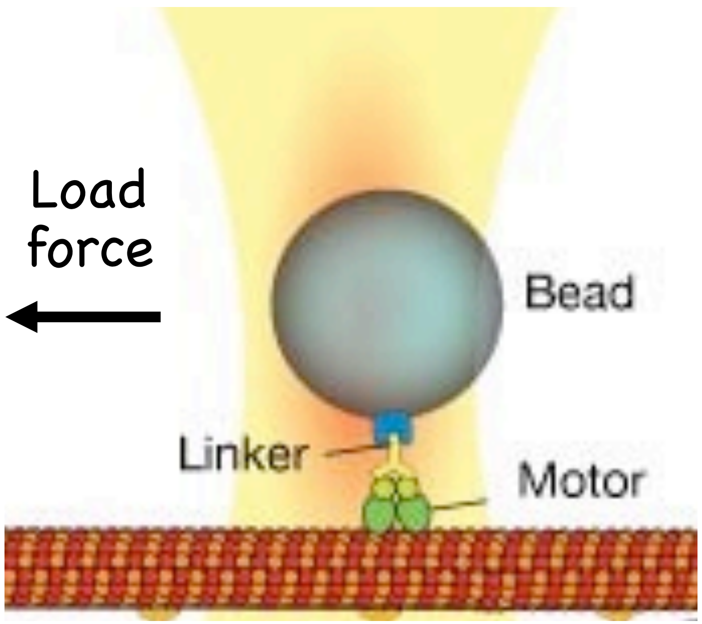
Force-velocity curve (kinesin)

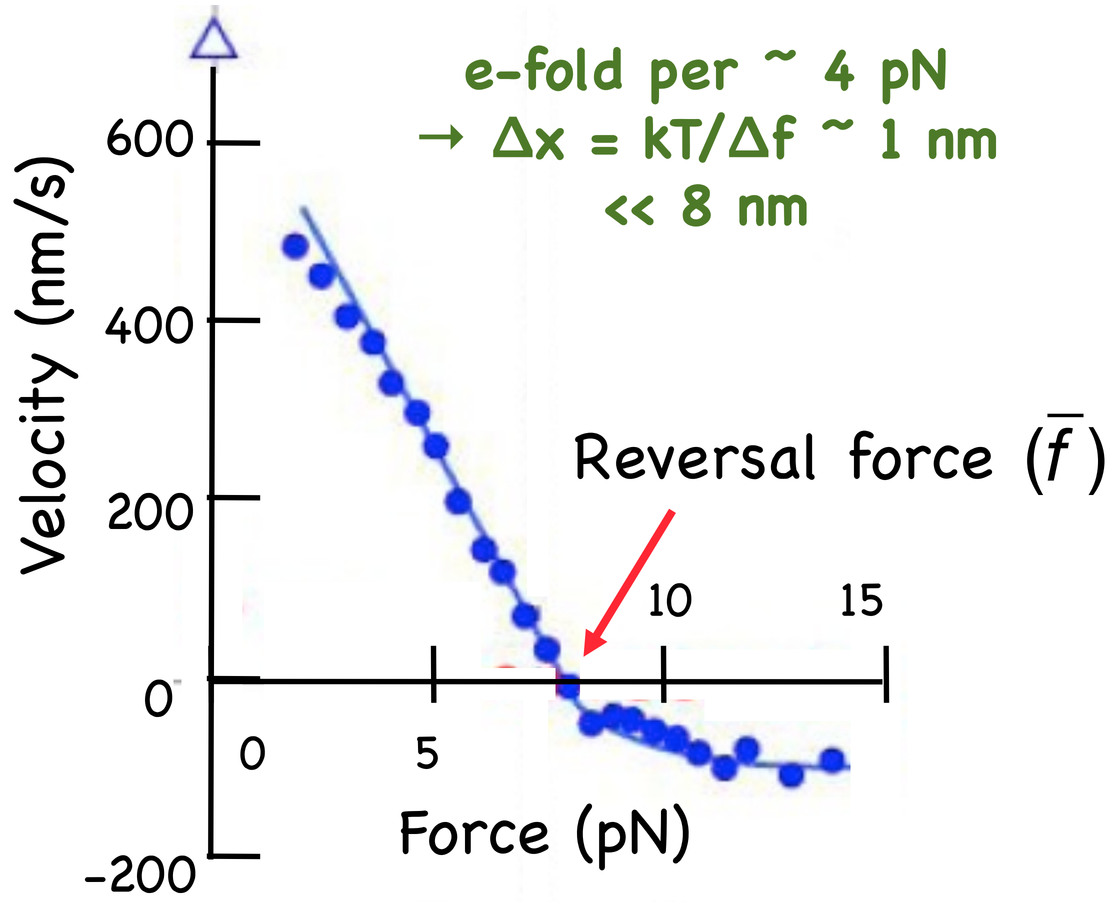




Force-velocity curve (kinesin)







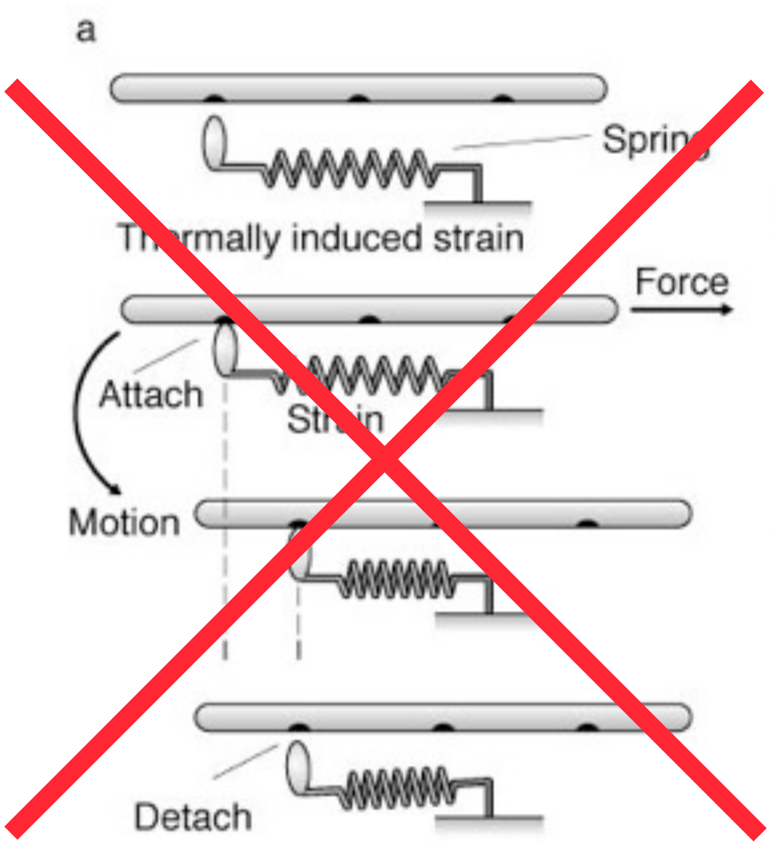
e-fold per ~ 4 pN

→ $\Delta x = kT/\Delta f \sim 1$ nm

$\ll 8$ nm

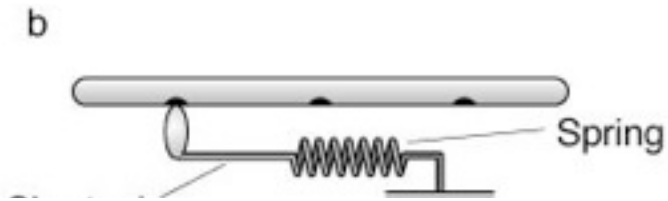
Carter & Cross 2005

Thermal ratchet model

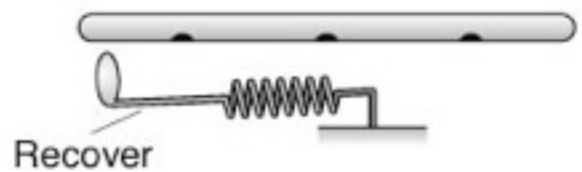


Huxley 1957
Cordova et al. 1992

Powerstroke model



small distance to transition state assures reasonable speeds even at high forces

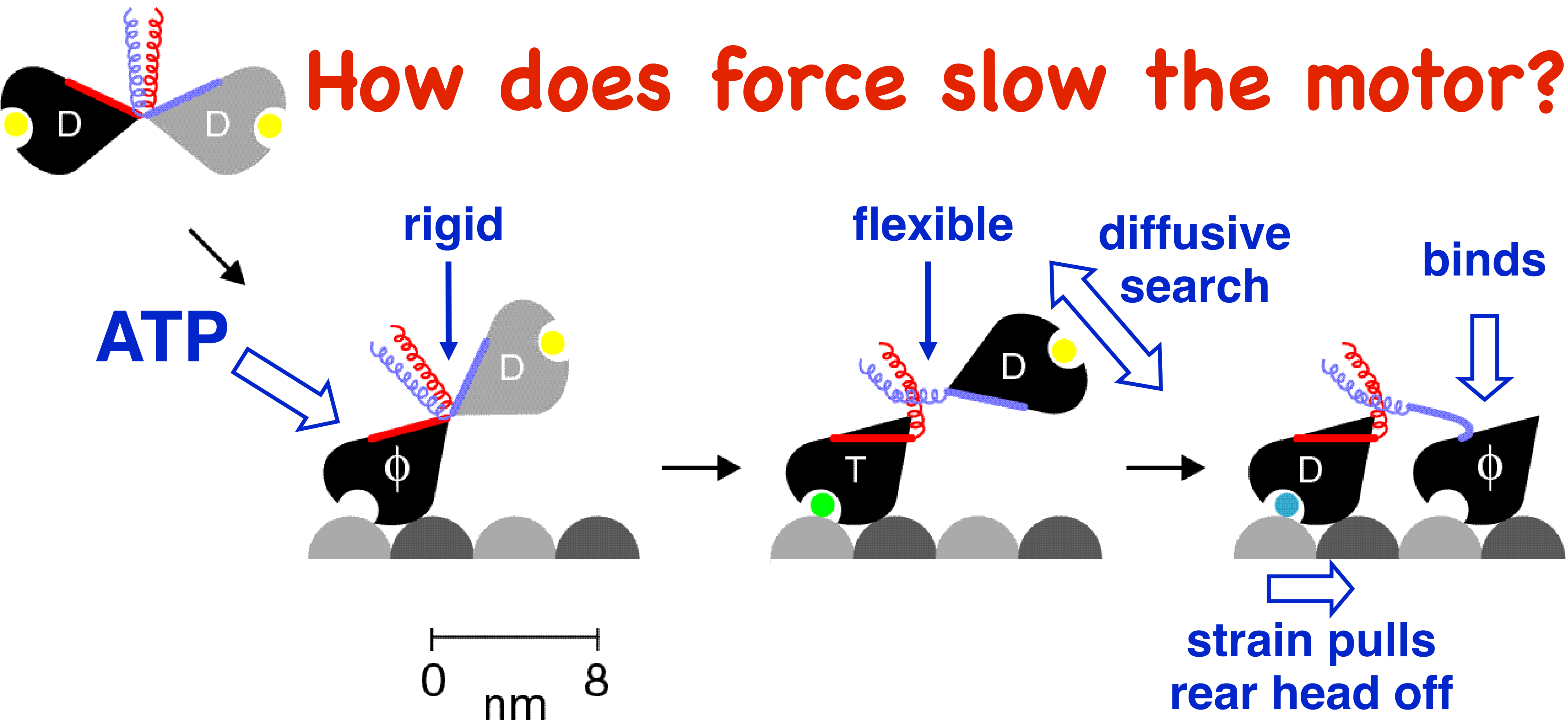


Eiesenberg & Hill 1978
Parmeggiani et al. 1999

High processivity (kinesin does not let go)

$\Rightarrow$ at least two binding sites

Hand-over-hand model



Hancock & Howard 1998, 1999; Schief & Howard 2001; Schief et al. 2004

Outline

1. Single-molecule techniques can be used to study movement of purified motor proteins

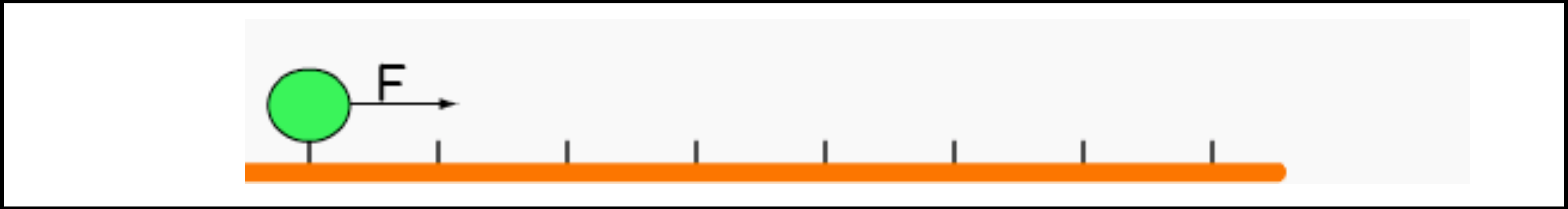
2. Role of fluctuations in the motor reaction

3. **Protein friction** limits motor speed and efficiency

4. Force gating of motor proteins: active mechanical circuits underlying cell motility

Friction …

… resists the relative motion of two bodies in contact.



Friction arises from the force necessary to deform and break adhesive bonds.

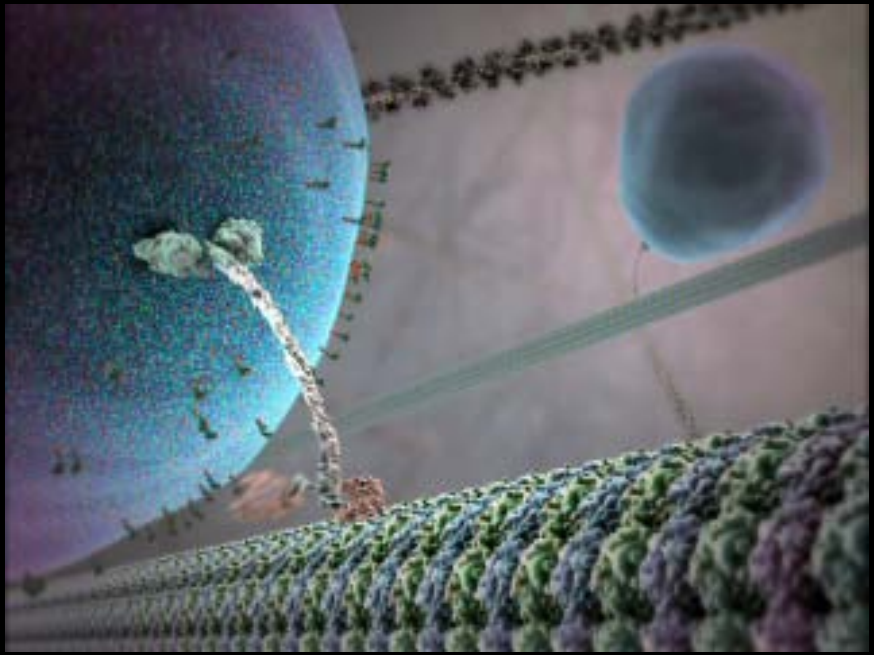
When a bond breaks, the energy stored in its deformation is dissipated

1) Protein Friction ...

... is especially important for motor proteins

ATP -> ADP + P + Energy

work ↙ ↘ friction



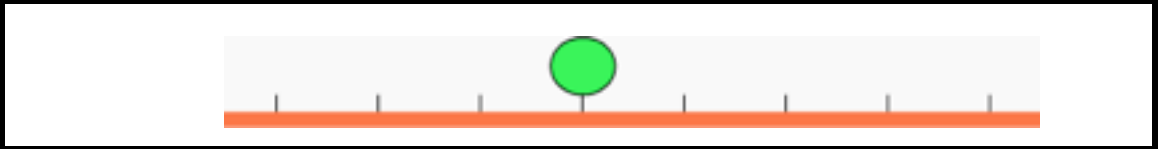
Harvard university

Friction forces acting on motors have not been measured

How they depend on speed are unknown

2) Protein Friction ...

... is related to diffusion



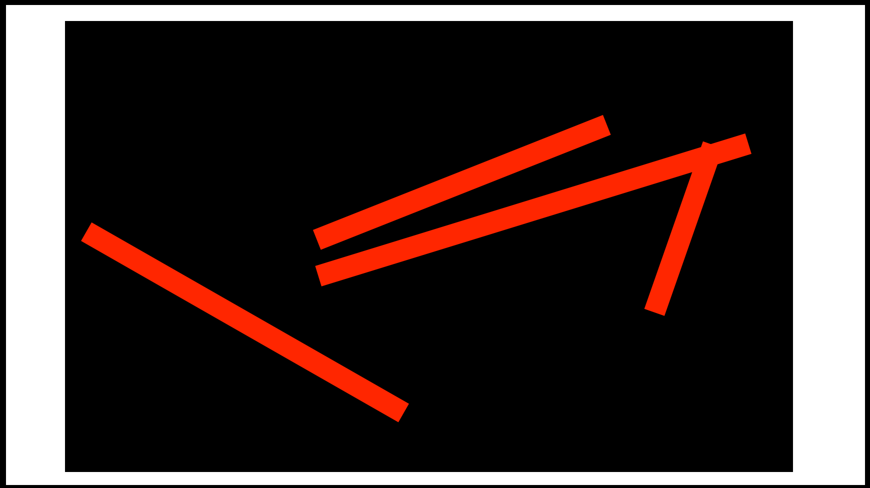
Diffusion

$$D = \frac{k_B T}{\gamma}$$

$$F_{friction} = -\gamma v$$

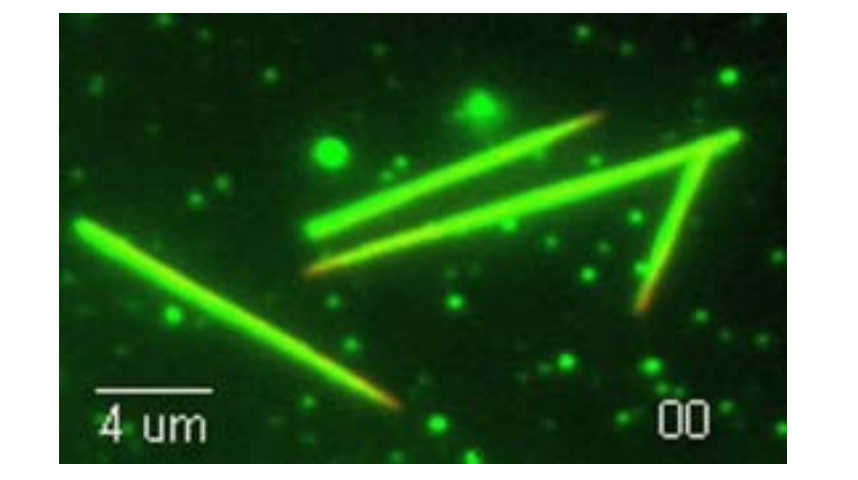
Friction

The budding yeast kinesin-8 (Kip3p) is a model system to study protein friction



Varga et. al 2006

The budding yeast kinesin-8 (Kip3p) is a model system to study protein friction

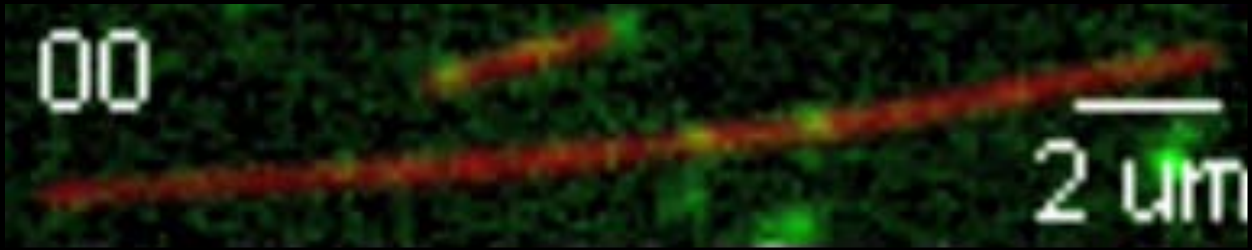


Varga et. al 2006

Microtubule depolymerase

With key role in microtubule length regulation

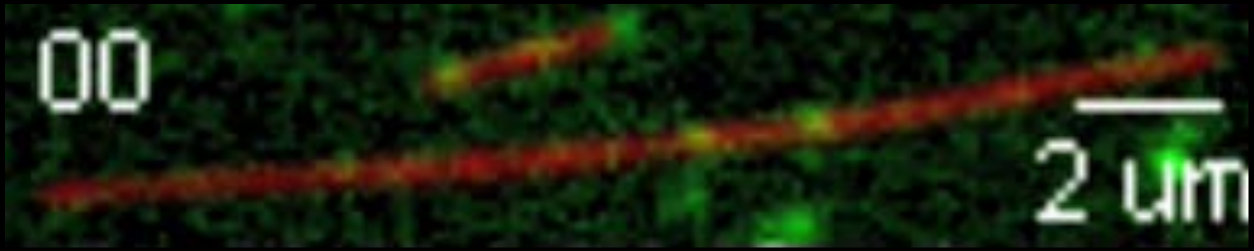
In ATP: Highly processive plus end directed motor



Varga et. all 2006

$$v = 3\,\mu\mathrm{m}/\mathrm{min}$$

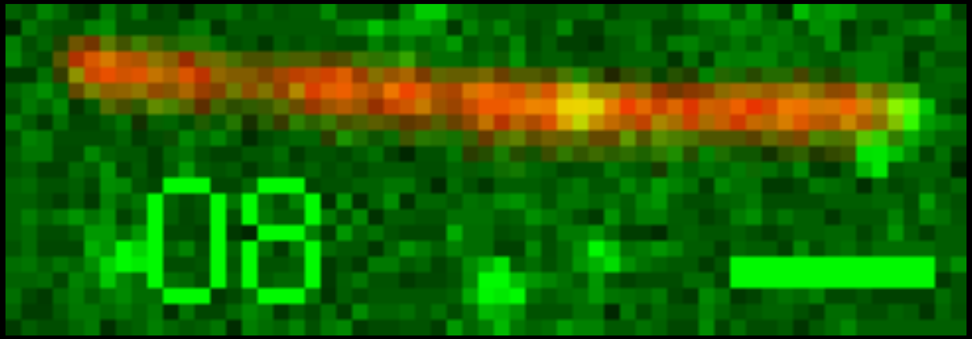
In ATP: Highly processive plus end directed motor

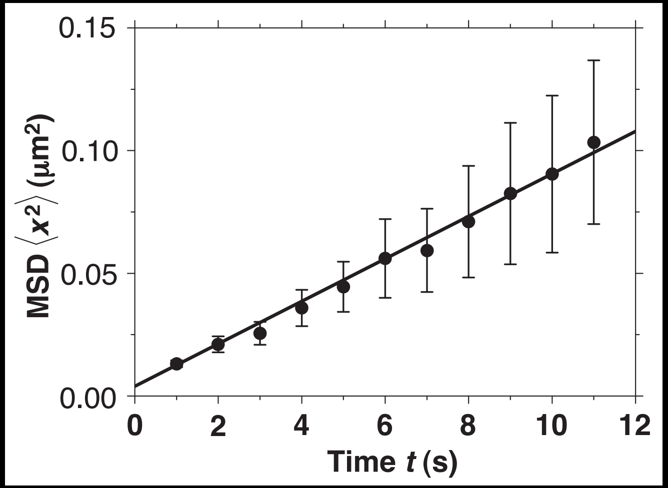


$$v = 3\,\mu\text{m}/\text{min}$$

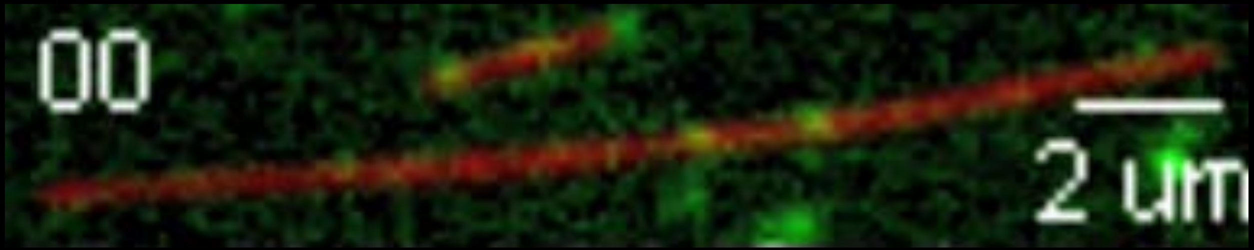
Varga et. al 2006

In ADP: Kip3p diffuses on microtubules





In ATP: Highly processive plus end directed motor



Varga et. al 2006

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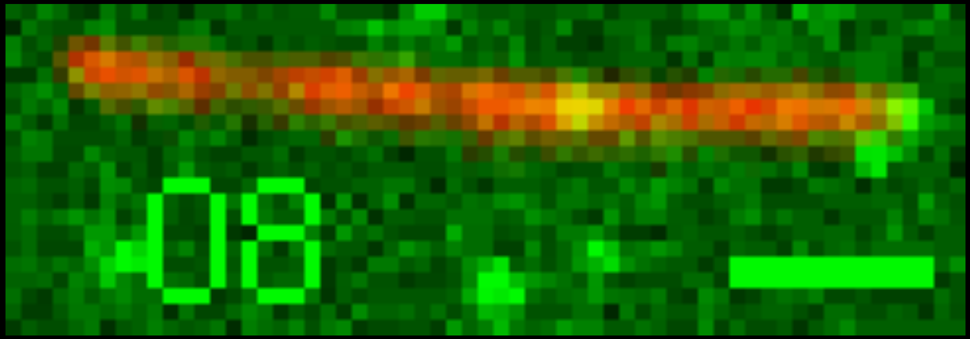
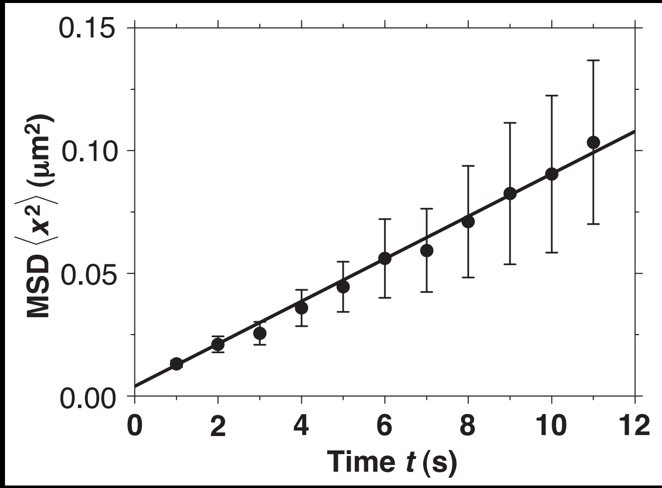
In ADP: Kip3p diffuses on microtubules

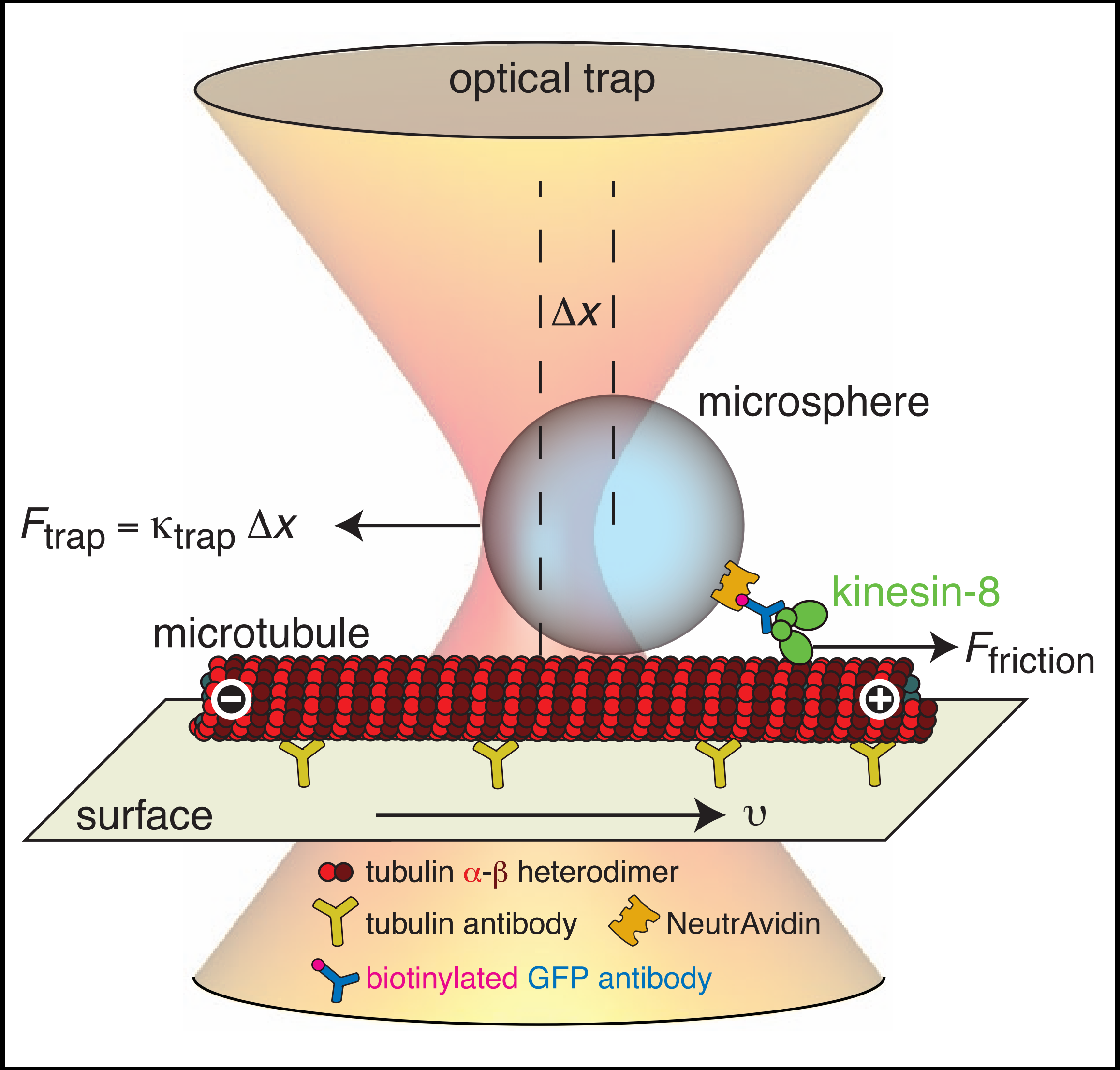




$$\gamma = \frac{k_B T}{D} \qquad \gamma = 0.95 \pm 0.11\,\mu\mathrm{Ns}/\mathrm{m}$$

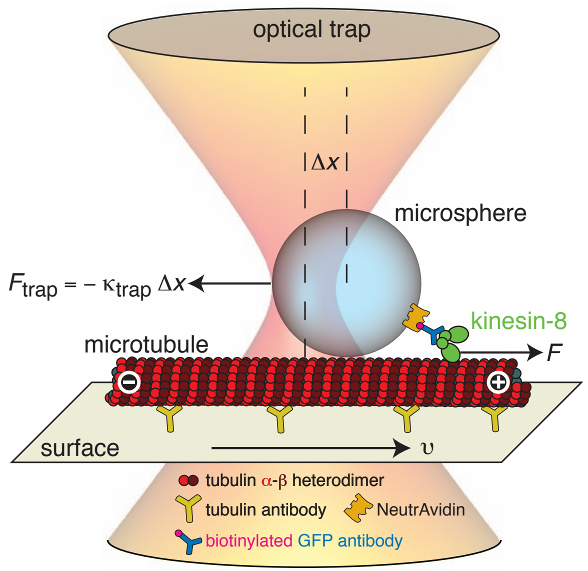
$$v \approx 1\,\mu\mathrm{m}/\mathrm{s} => F = \gamma v \approx 1\,\mathrm{pN}$$

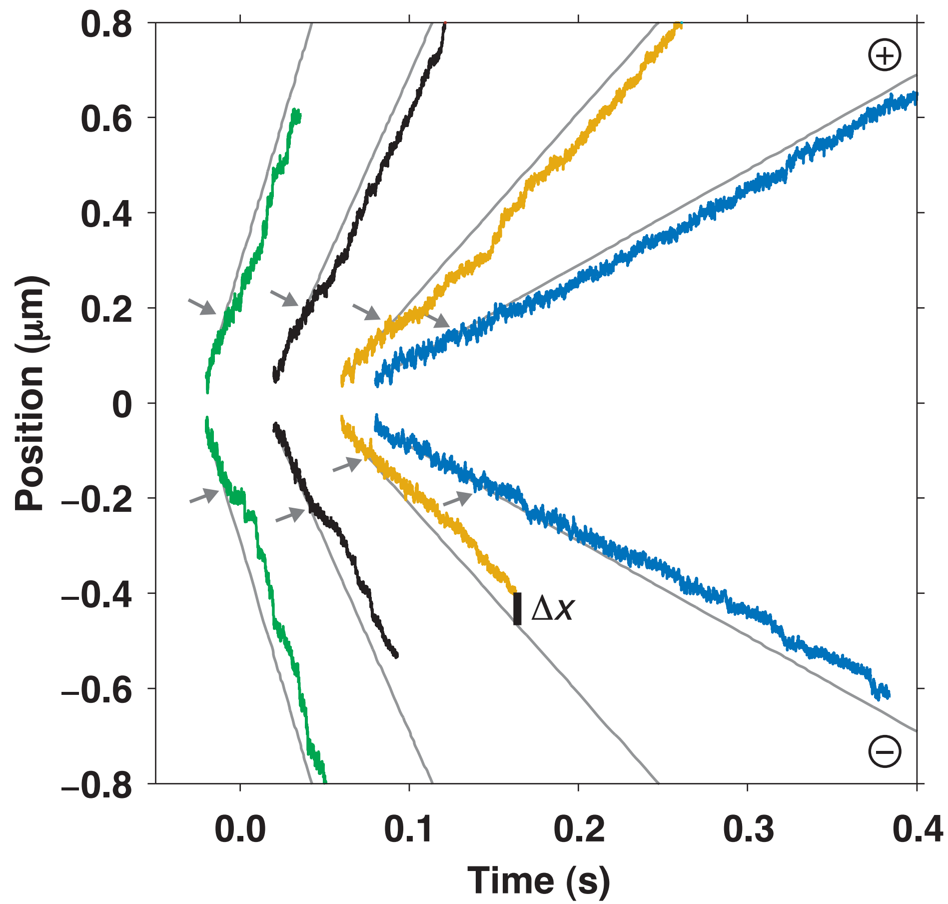
The friction measurement:



Bormuth et al., Science 2009

Optical tweezers can measure friction force





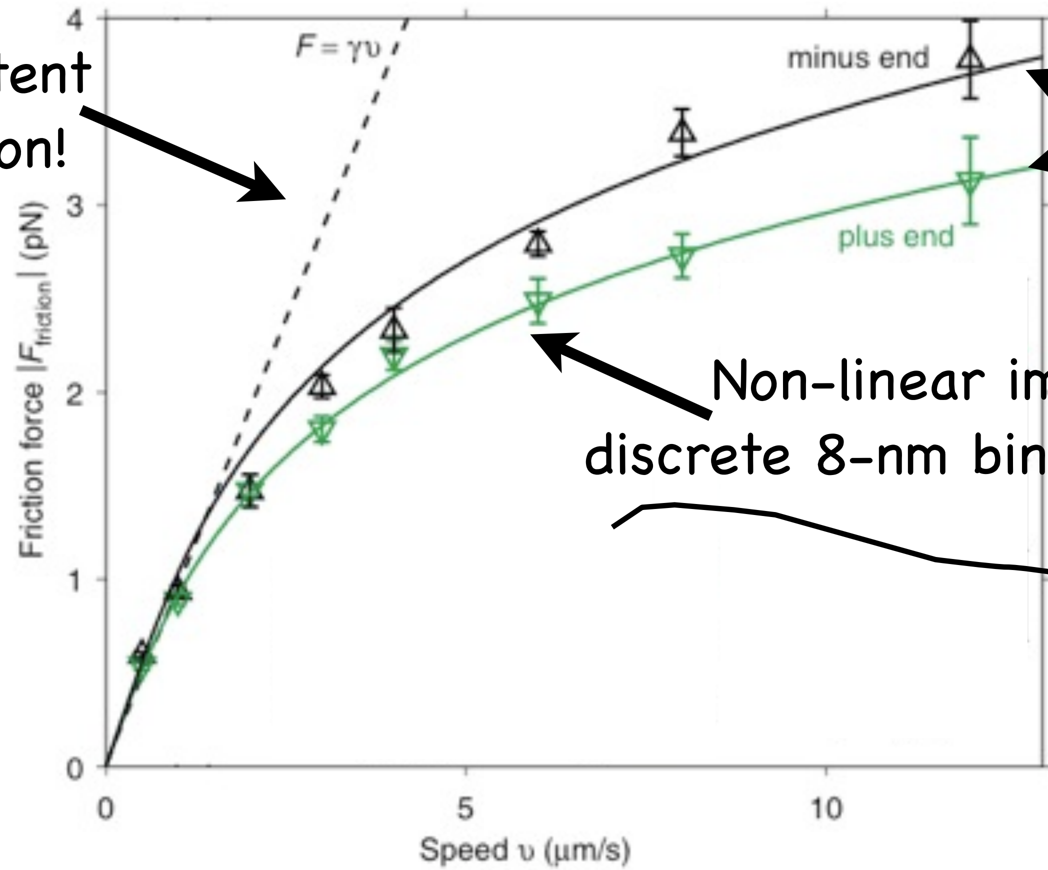
Bormuth et al., Science 2009

Kinesin-8 has non-linear protein friction

Slope consistent with diffusion!

-> big bead is not contributing to the friction

-> consistent with being close to thermal equilibrium

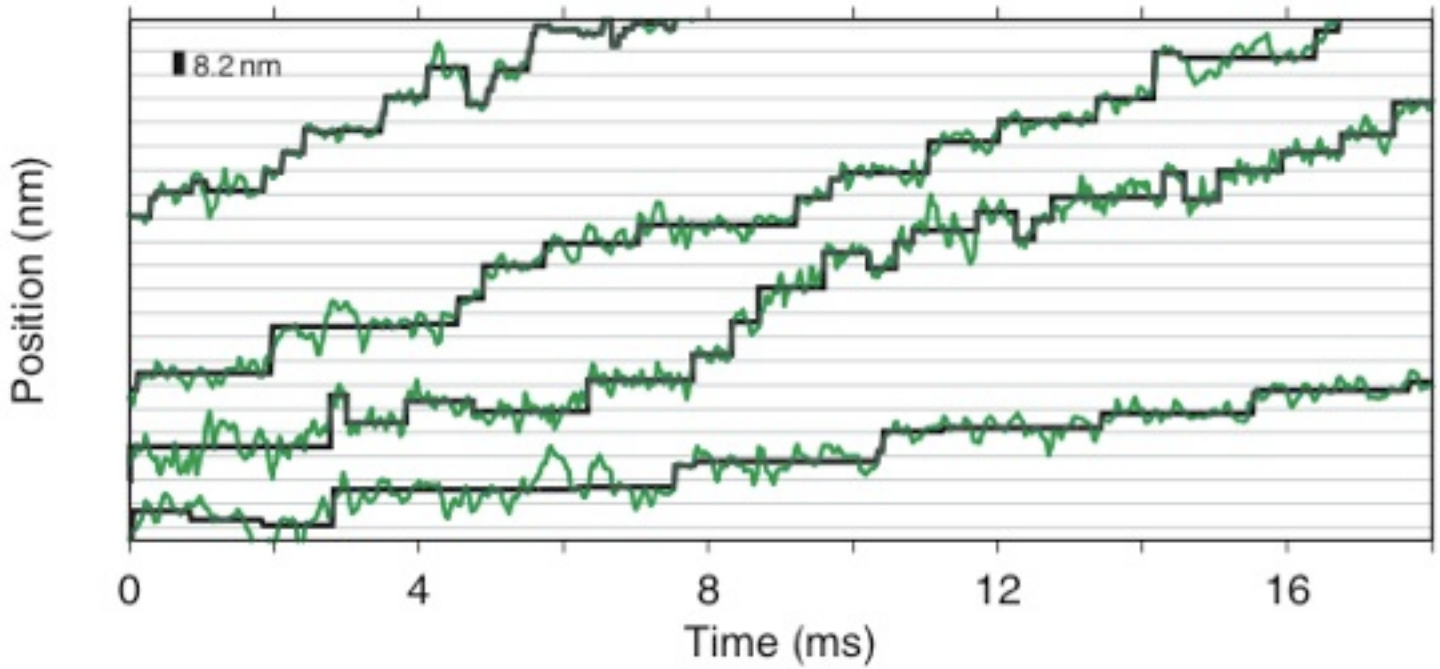


Asymmetry!

Non-linear implies discrete 8-nm binding sites!

Bormuth et al., Science 2009

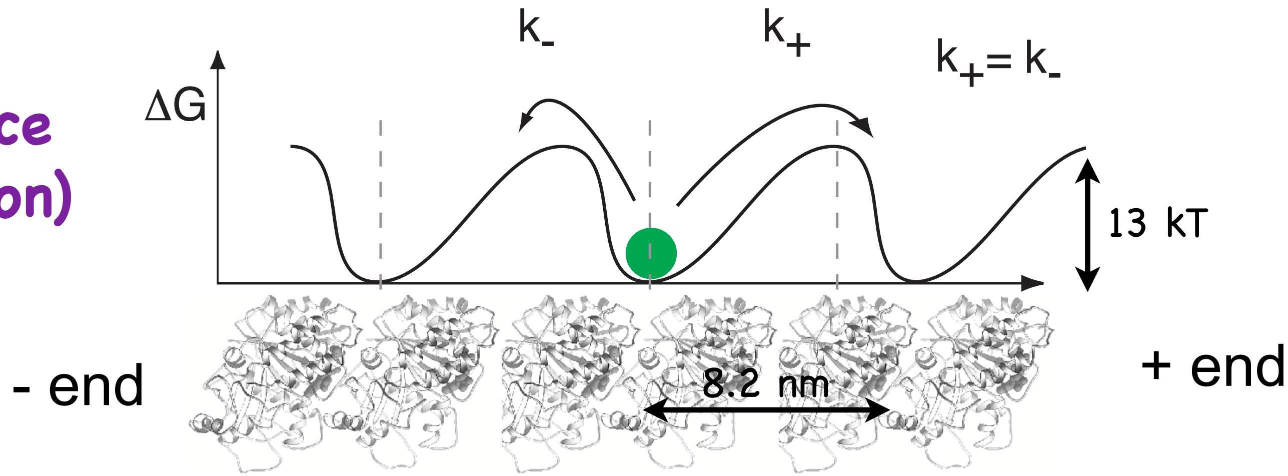
Frictional slipping is in 8-nm steps



Bormuth et al., Science 2009

Diffusion and friction can be described as motion in a periodic potential

No force (diffusion)

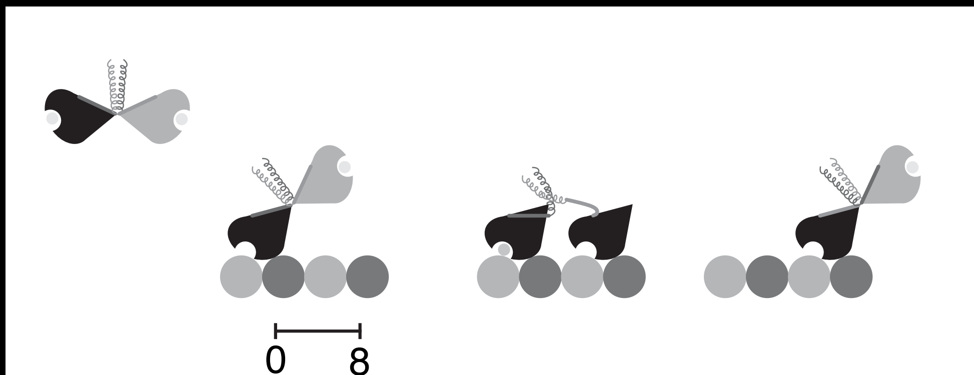


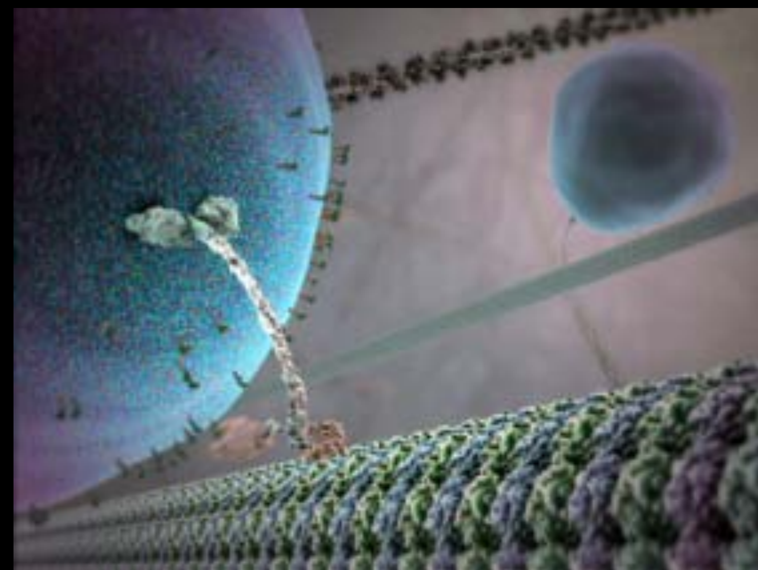
Hopping rates: 1 to 10,000/s (depending on kinesin and nucleotide state)

Bormuth et al., Science 2009

How does the measured friction limit the motility of kinesin when it is driven by ATP-hydrolysis?

Picture of a motor as a force generator limited by a damping element

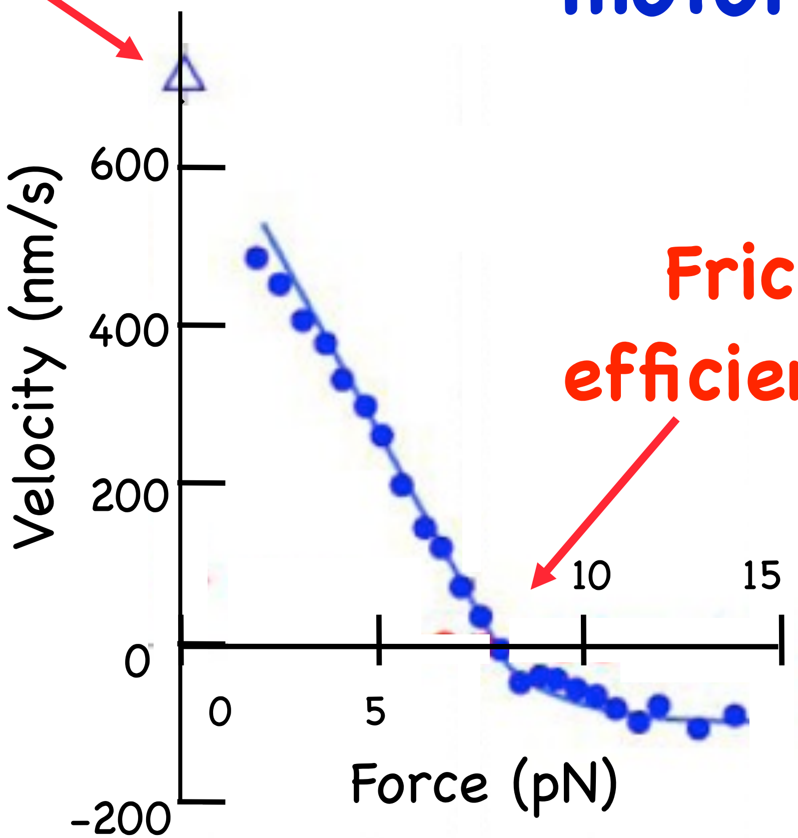




sticky feet

Friction helps to understand the force-velocity curve of motor proteins

Friction limits maximum speed

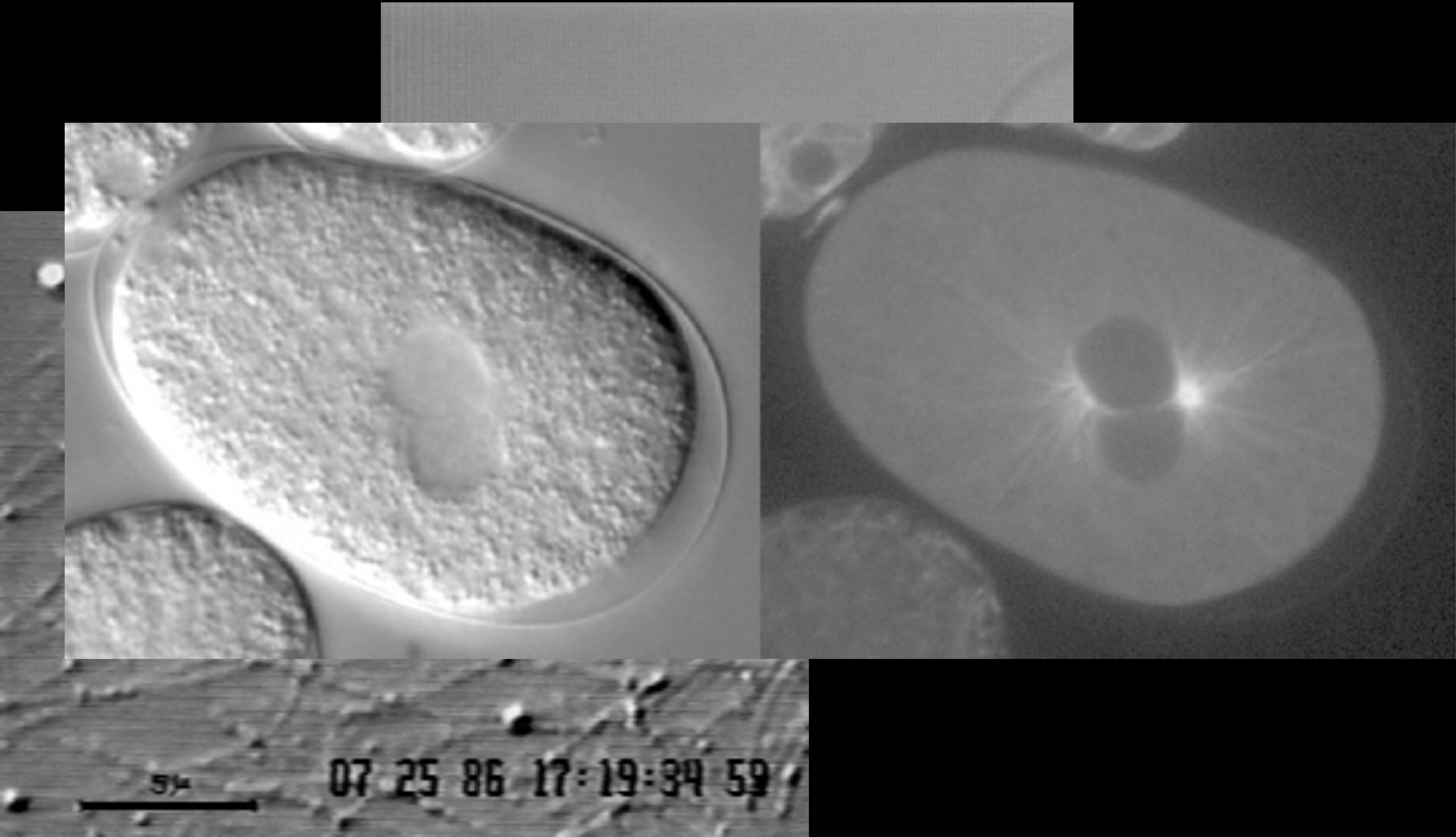


Friction limits efficiency (slipping)

But ... this is boring!

Carter & Cross 2005

Mechanical switching and oscillations in cells



Outline

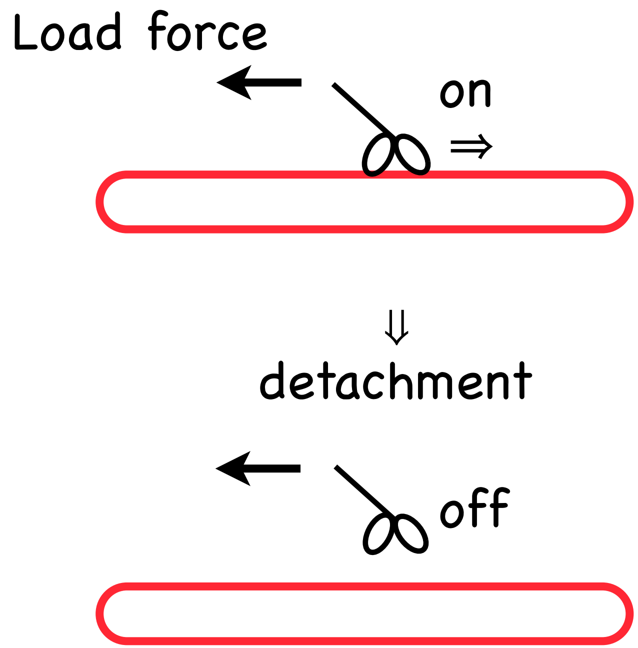
1. Single-molecule techniques can be used to study movement of purified motor proteins

2. Role of fluctuations in the motor reaction

3. Protein friction limits motor speed and efficiency

4. **Force gating of motor proteins**: active mechanical circuits underlying cell motility

Force-accelerated detachment of motors

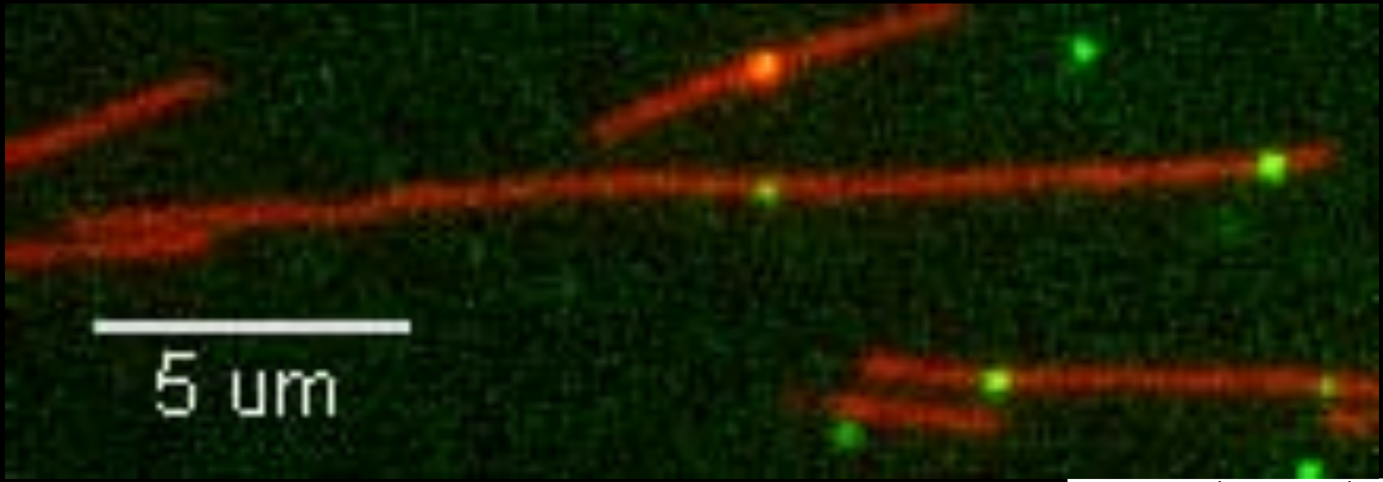


Can give rise to **negative friction**

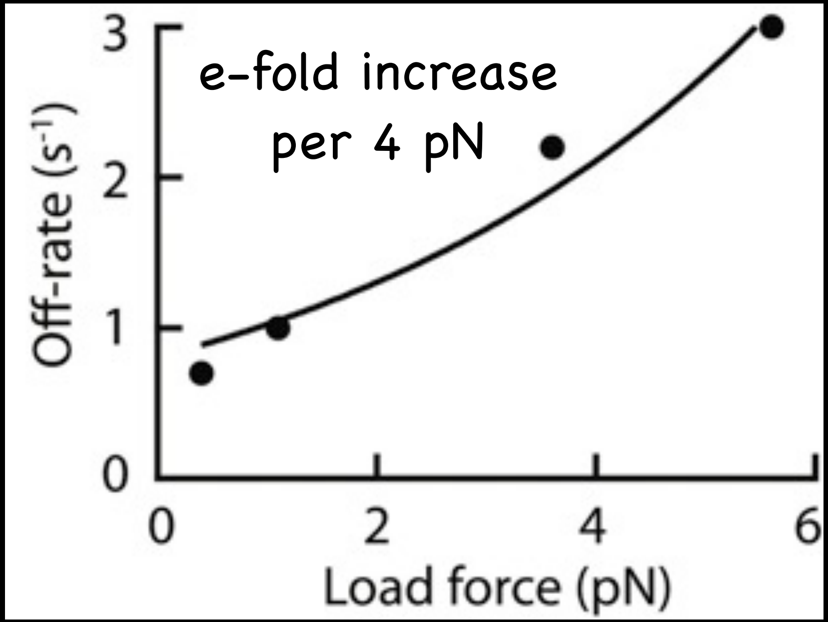
Can lead to **switching, oscillations!**

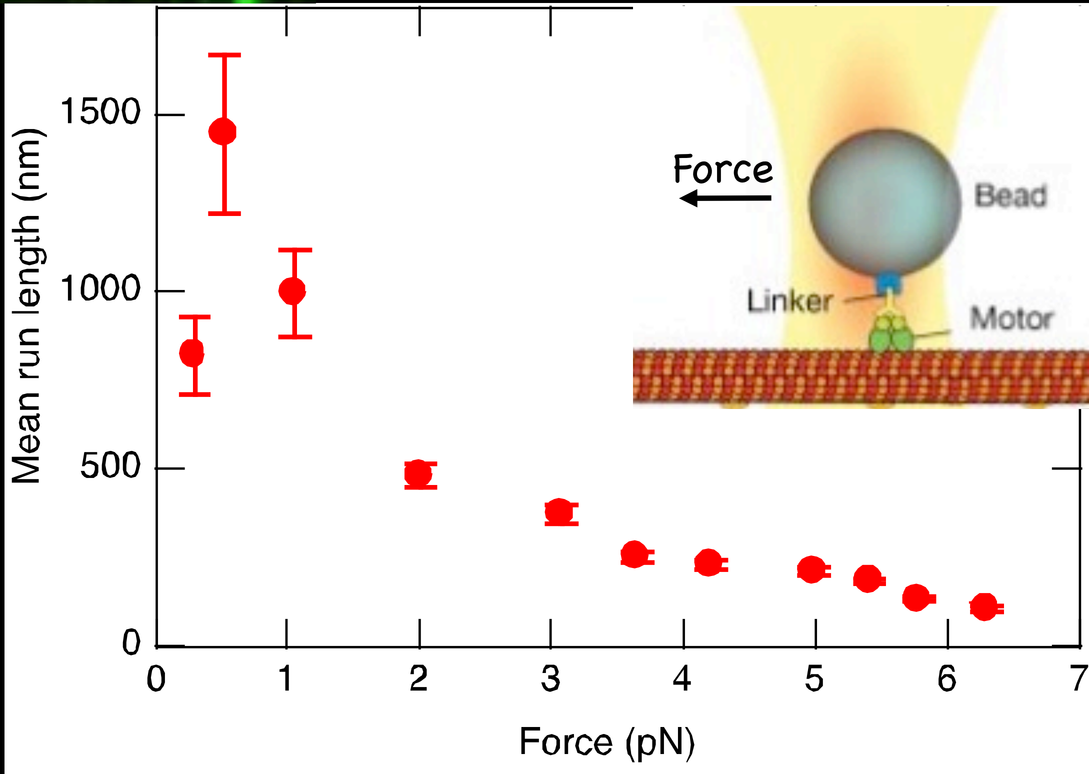
Howard, Ann. Rev. Biophys. 2009

Load-accelerated dissociation of kinesin-1



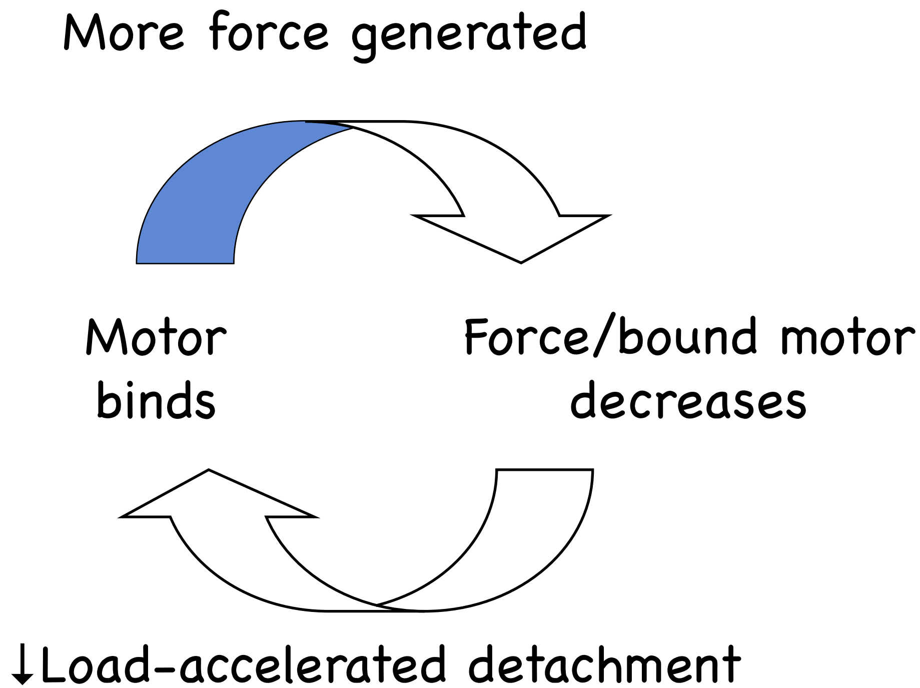
Schnitzer et al. 2000





Load-accelerated dissociation

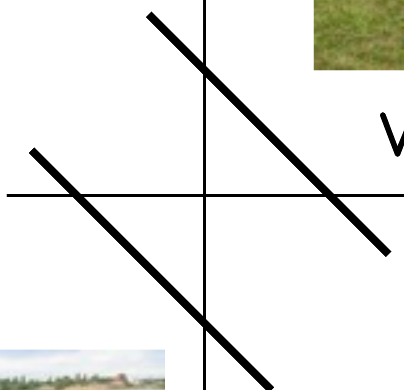
→ Positive feedback

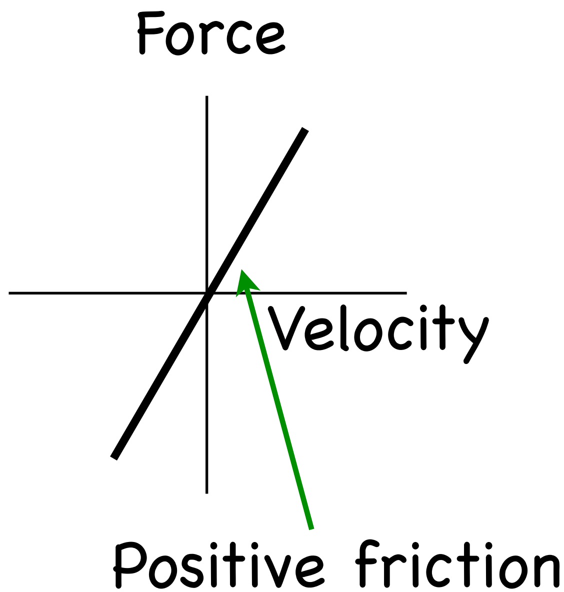


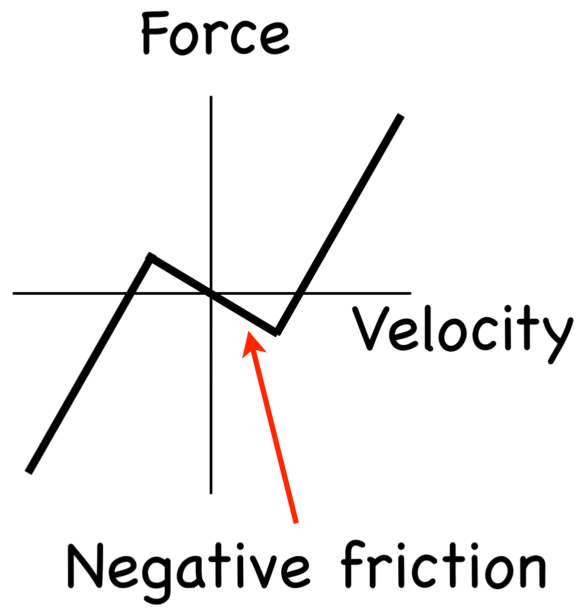
Howard, Ann. Rev. Biophys. 2009

Load-accelerated dissociation

→ Negative friction







⇓

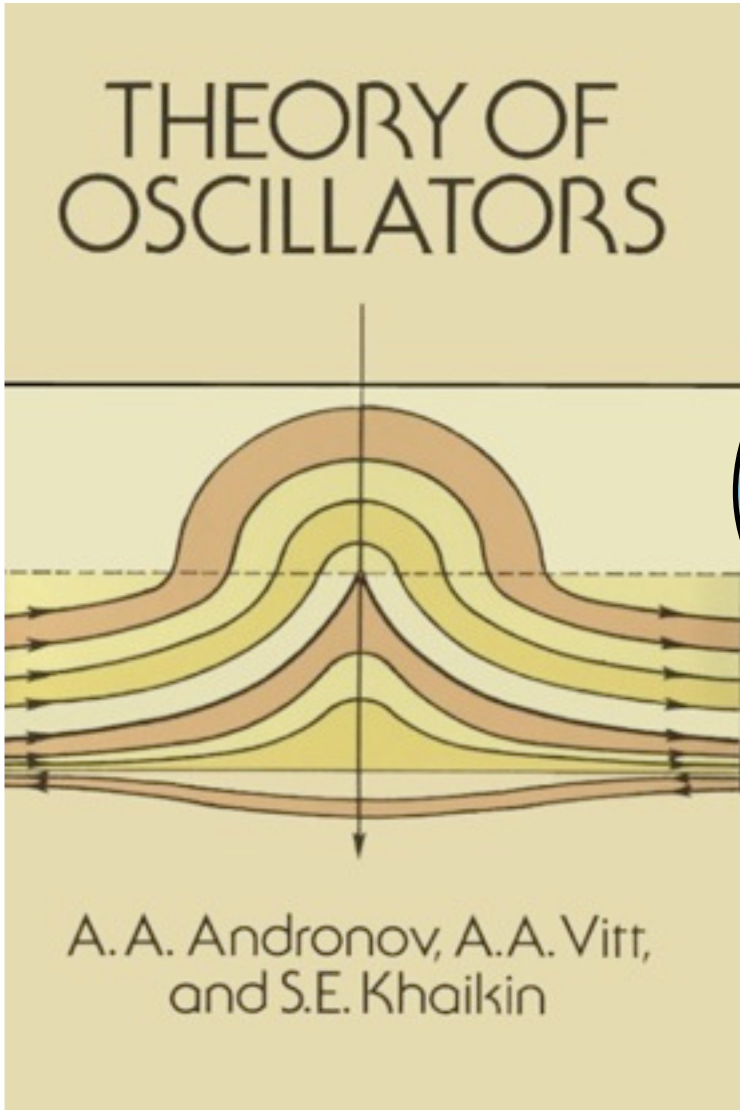
switching, oscillations

(Julicher & Prost, 1995,1997)

Mechanical transistor!

Howard, Ann. Rev. Biophys. 2009

Theory of dynamical systems



First edition, ~1930

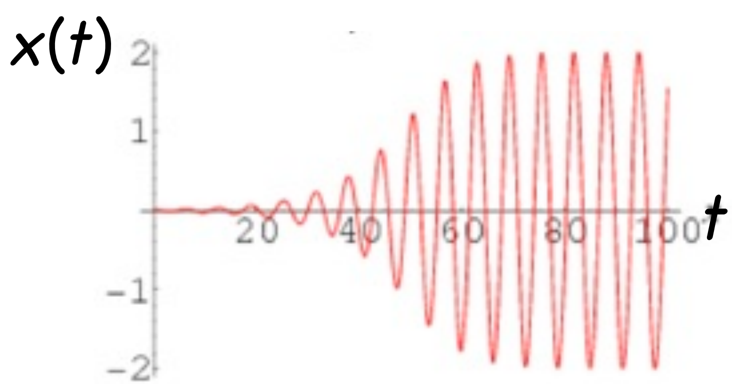
What do you need for a self-sustained, stable oscillation?

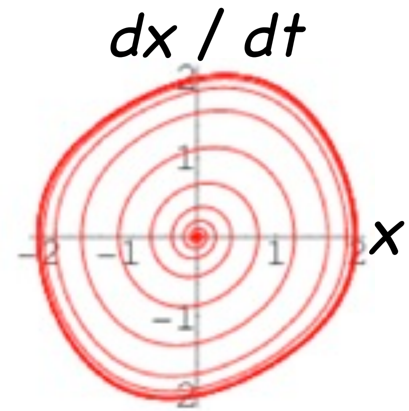
Negative friction

$$+ \gamma \frac{d}{dt} x$$

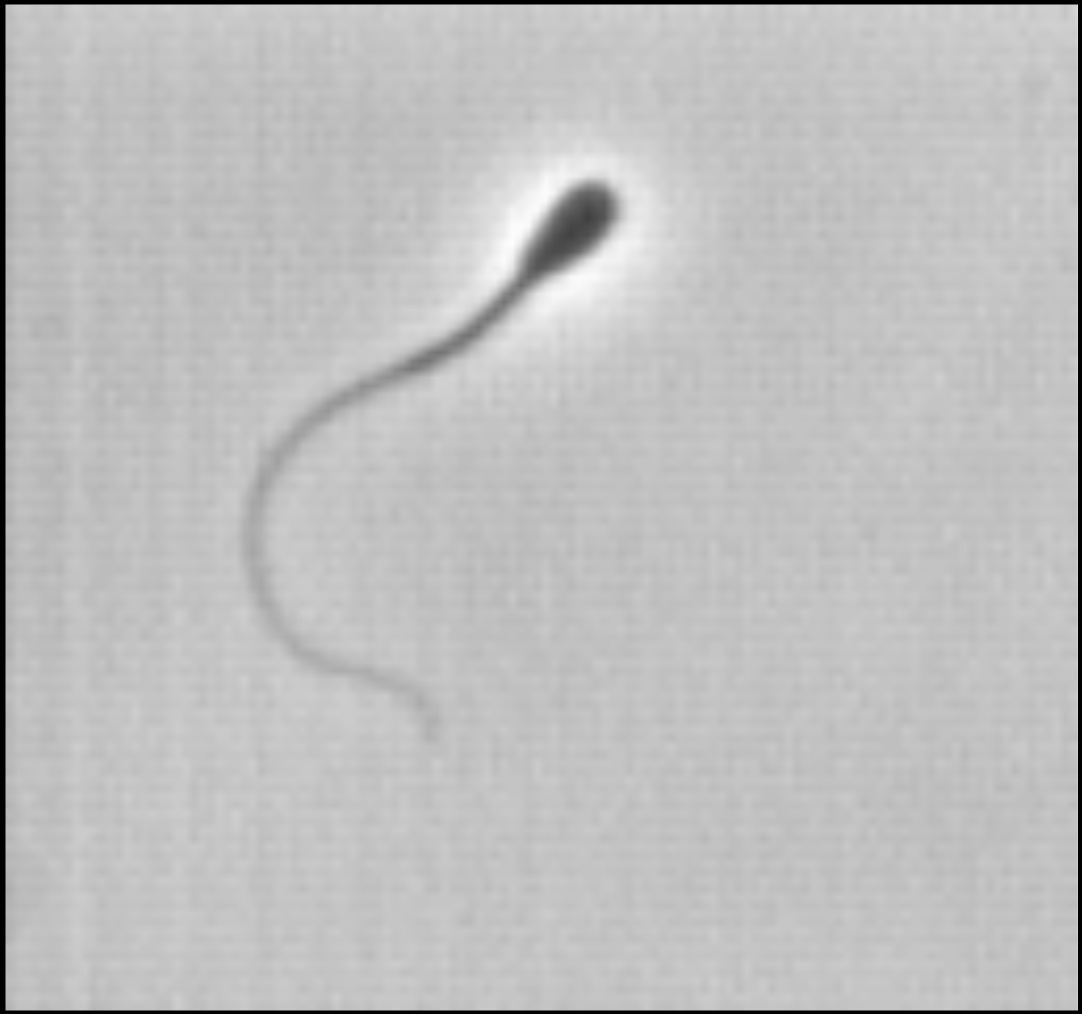
Non-linearity

$$m \frac{d^2}{dt^2} x - \varepsilon \left(A - x^2 \right) \frac{d}{dt} x + \kappa x = 0$$





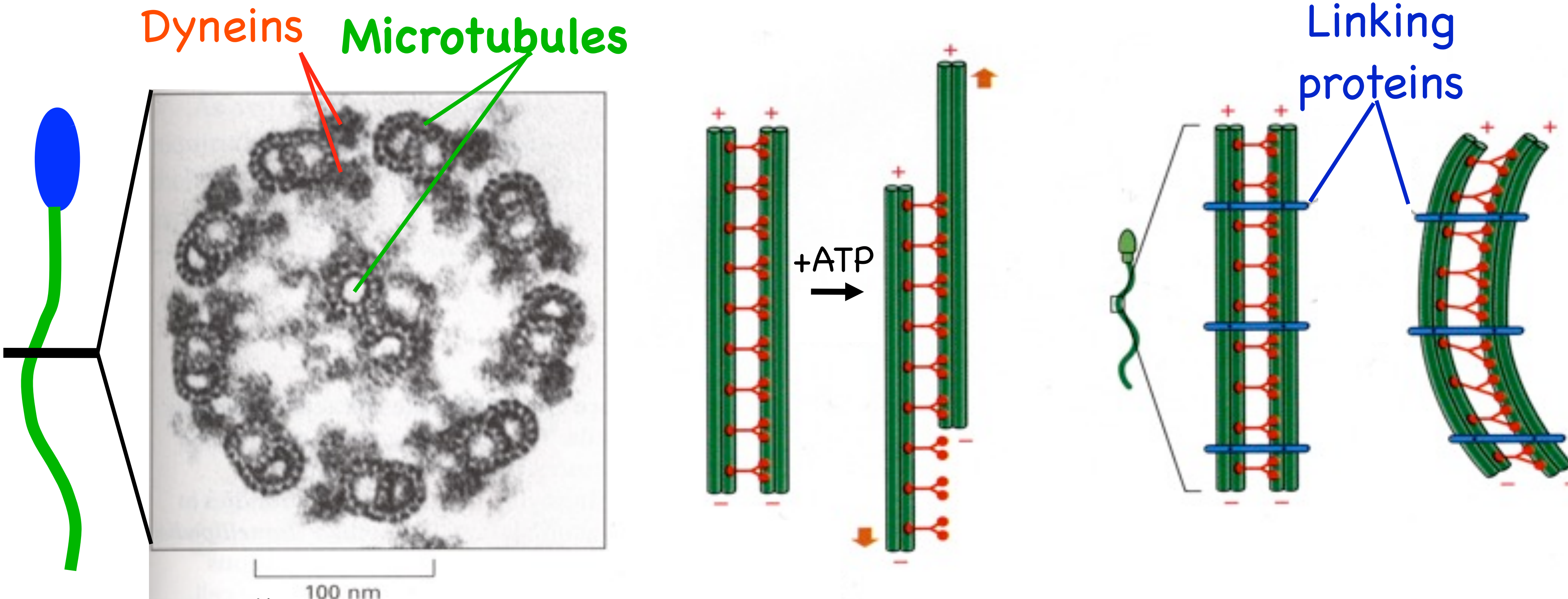
The flagellar beat



Bull sperm, L = 58 μm, f = 21 Hz, 22 °C

Riedel-Kruse, Hilfinger, Howard & Julicher 2007

Conversion of sliding to bending

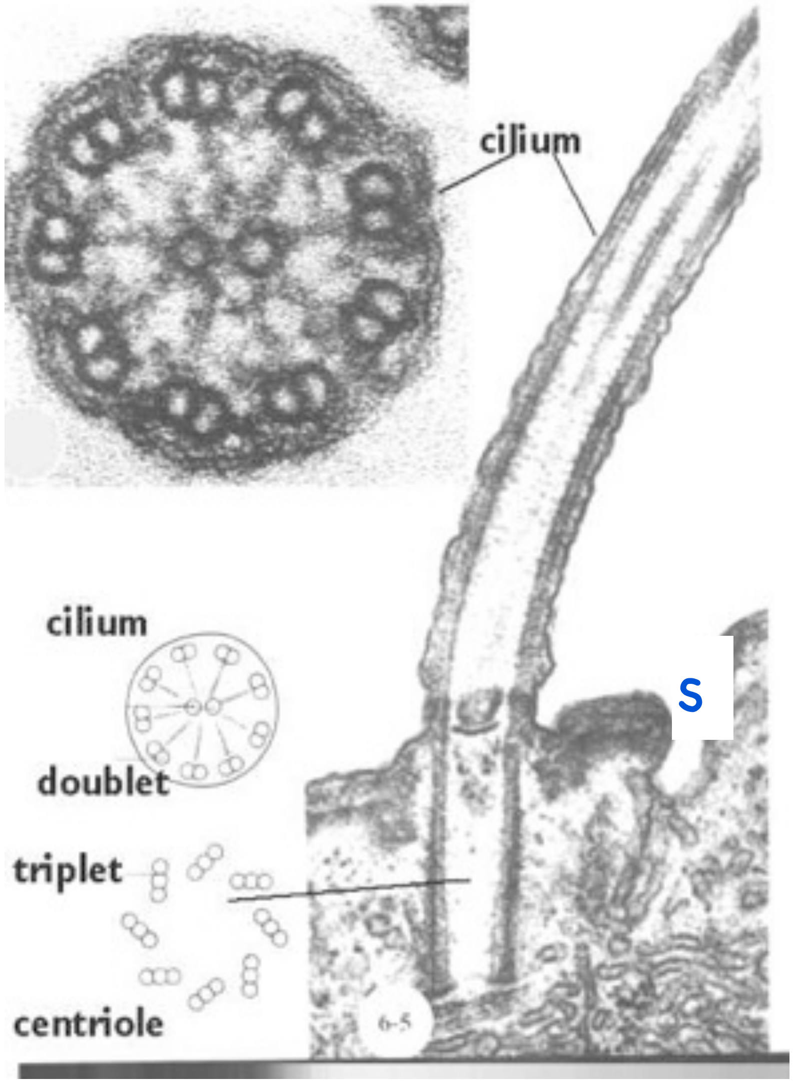


Tilney, 1973

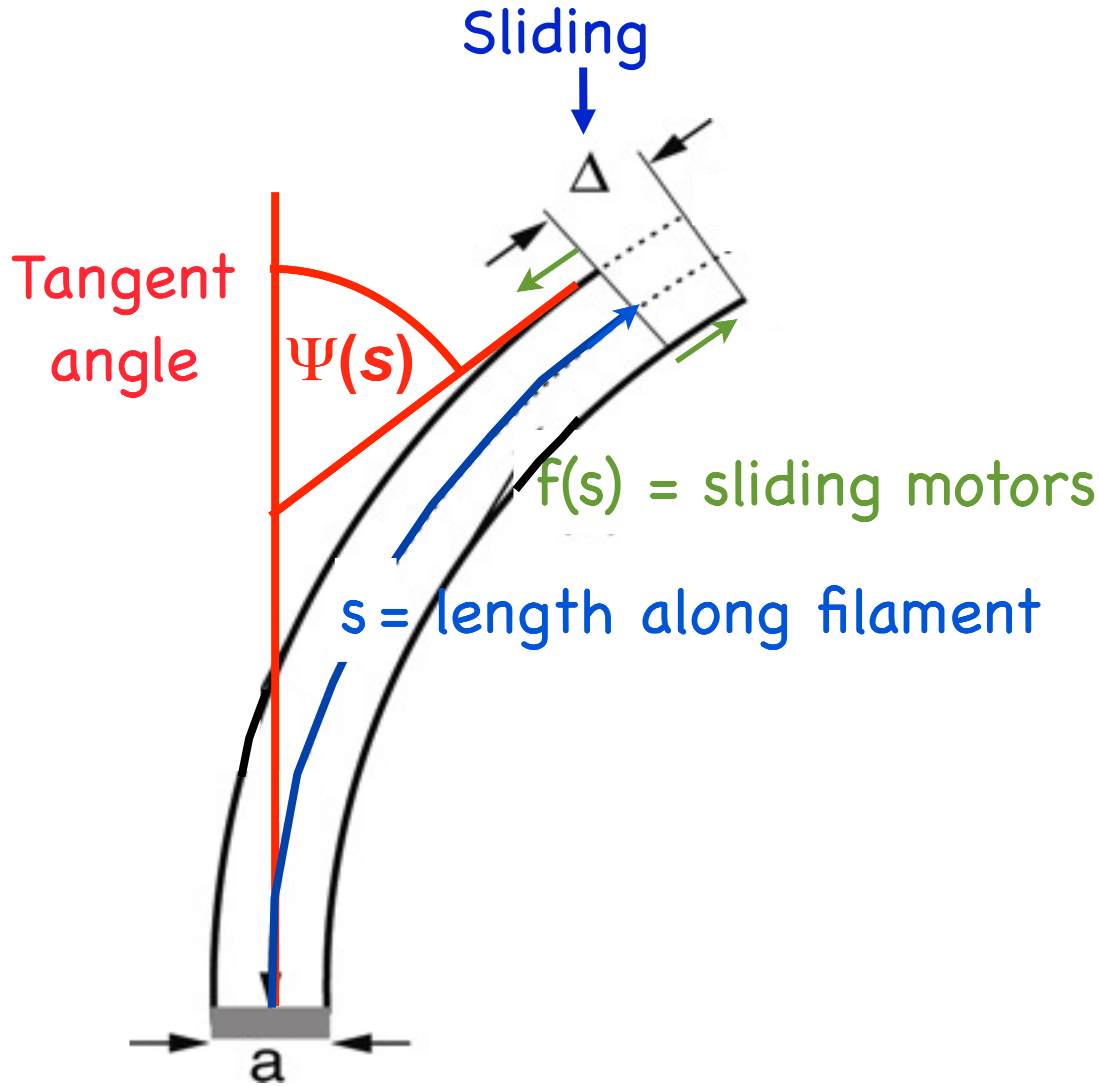
Alberts et al. Molecular Biology of the Cell

Two types of coordination
- across the section
- along the length

Bending and sliding



Gwen Childs, U. Arkansas



$$\Delta(s,t) = a \cdot \psi(s,t)$$

Flagellar oscillator

Oscillation requires

1. Negative friction (to supply energy) - load-accelerated detachment
2. Inertial term (delay) - delay of detachment
3. Elastic term (return to the center) - stiffness of the microtubules

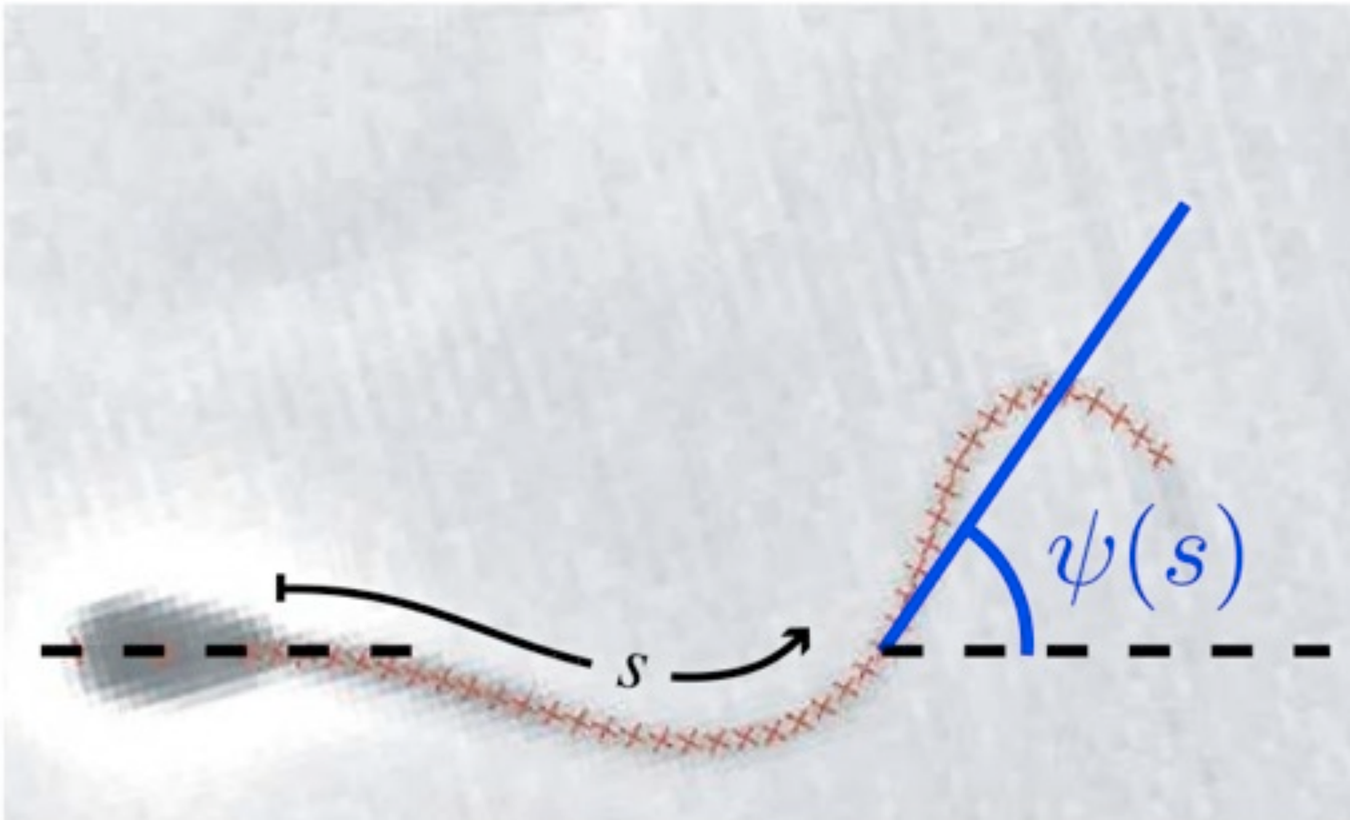
$\Downarrow$

"Sperm equation" $$a^2\chi \frac{d^2\tilde{\psi}}{ds^2}(s) = i\omega\xi_\perp \tilde{\psi}(s) + \kappa \frac{d^4\tilde{\psi}}{ds^4}(s)$$

Boundary conditions no external forces or torques (all internal)

Riedel-Kruse, Hilfinger, Howard & Jülicher 2007

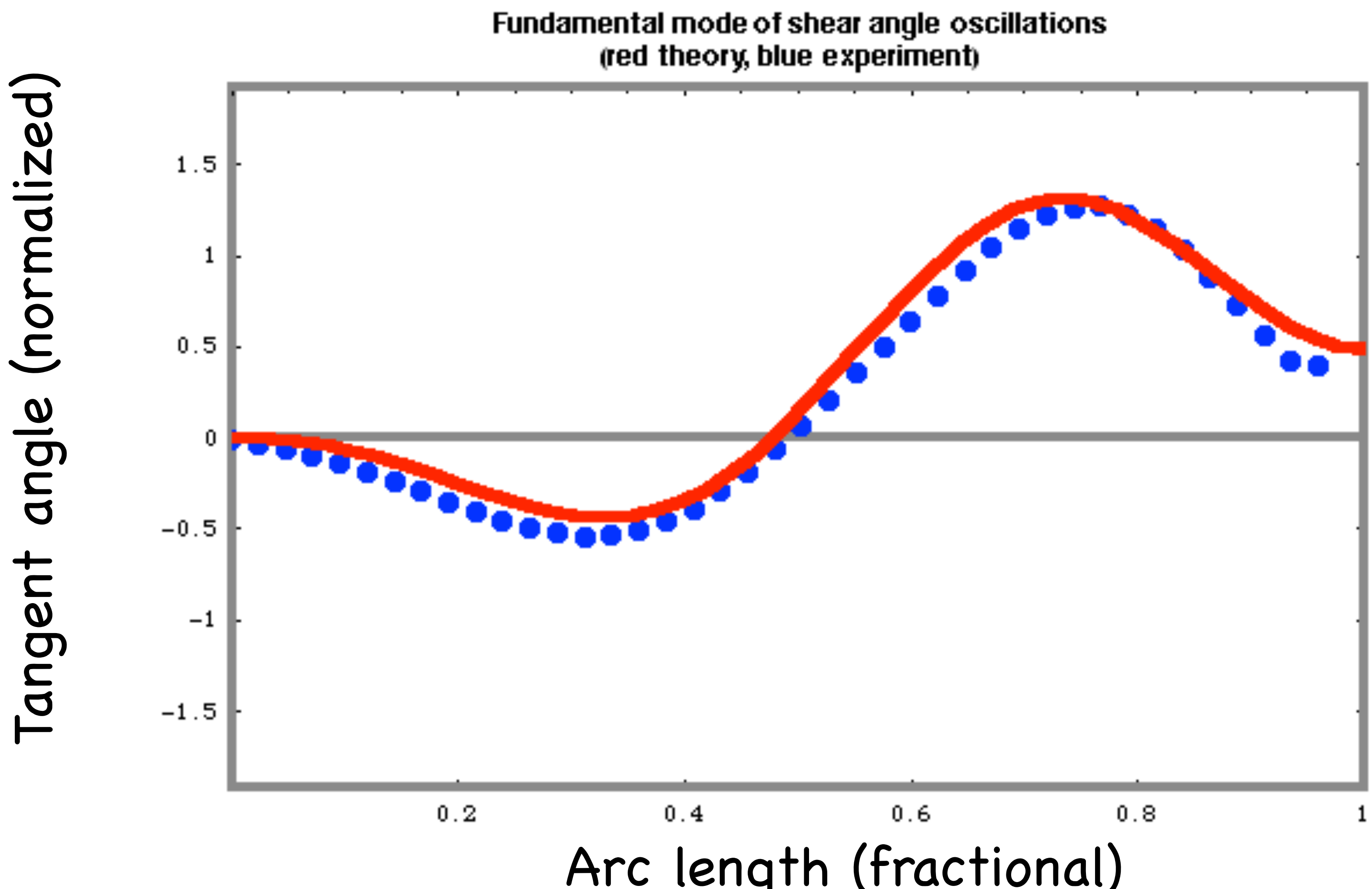
Determination of beat shape



Riedel-Kruse, Hilfinger, Howard & Jülicher 2007

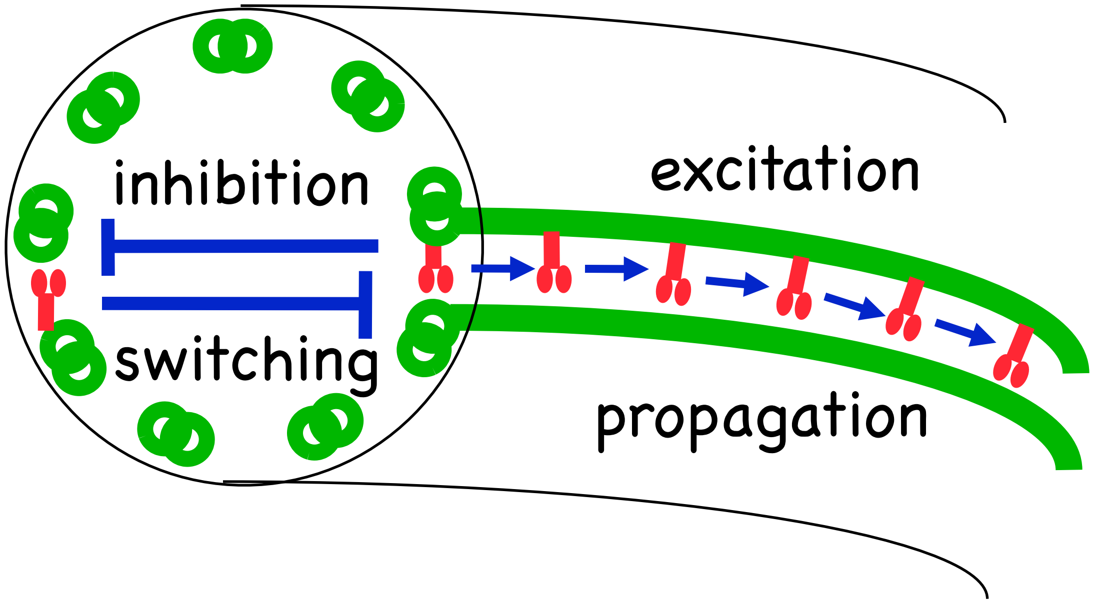
Agreement between theory and experiment

Clamped head



Riedel-Kruse, Hilfinger, Howard & Jülicher 2007

Mechanical signaling network in the axoneme



Howard, Ann. Rev. Biophys. 2009

Advantages of mechanical signaling over chemical signaling

1. can travel over large distances
2. signals move at the speed of sound (in the material)
3. feedback leads to coordination, switching and oscillation

Howard, Ann. Rev. Biophys. 2009

Summary

1. **Single-molecule techniques** can be used to study movement of purified motor proteins

2. Role of **fluctuations** in the motor reaction

3. **Protein friction** limits motor speed and efficiency

4. **Force gating of motor proteins:** active mechanical circuits underlying cell motility

mpipks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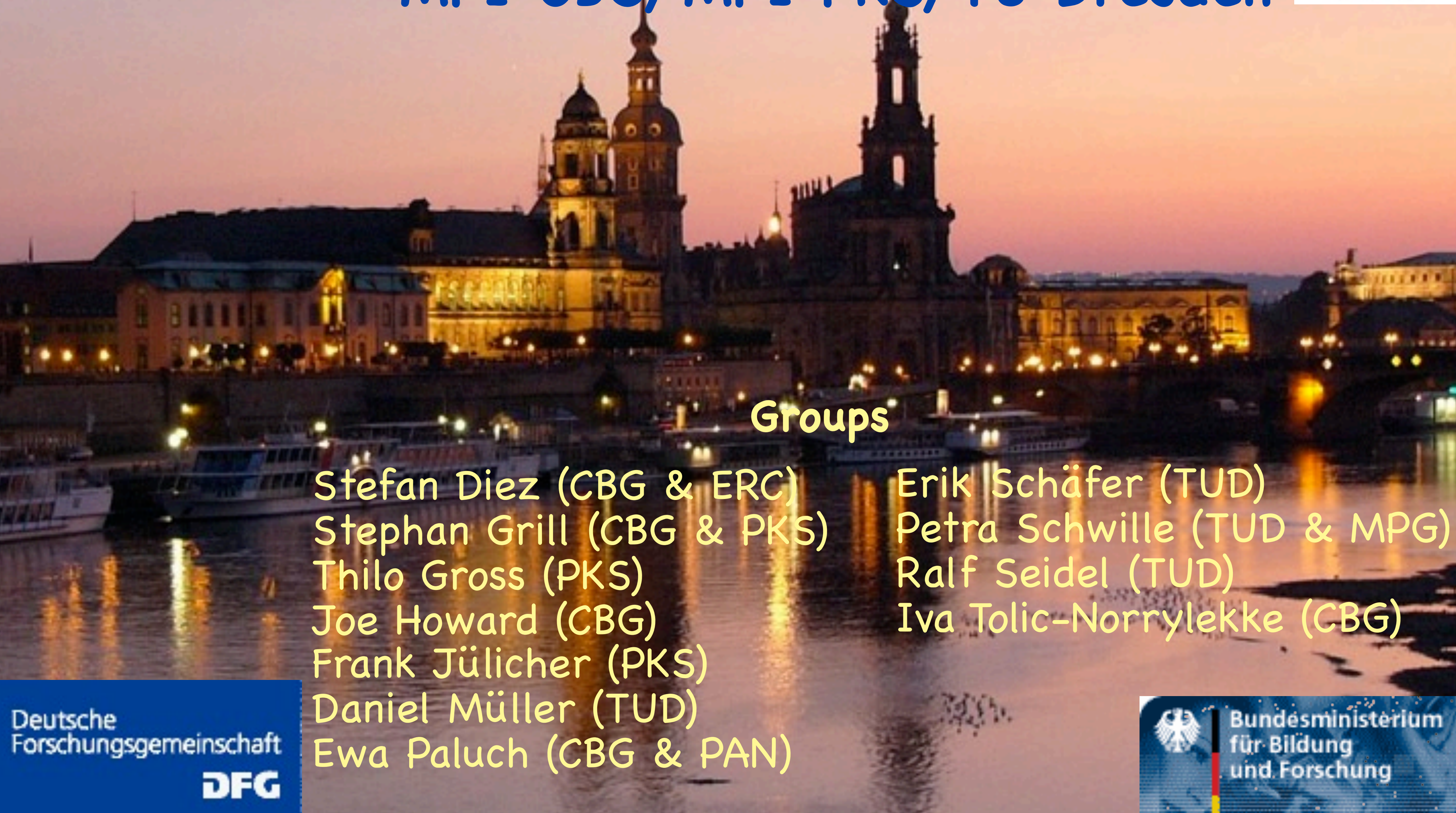
Physics of Biological Systems

MPI-CBG, MPI-PKS, TU Dresden



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